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Preface

We would like to present, with great pleasure, the inaugural volume-4, Issue-10, October 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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Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
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Treatment of landfill leachate through struvite precipitation and nitrogen removal bacteria and poly-phosphate bacteria (in-pots experiment)

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***Abstract*—** *Landfill leacheate is a type of wastewater which contains large amounts of nitrogen and phosphorus, therefore it needed to be treated before releasing to directly to the environment. The combination between struvite precipitation and nitrogen removal and poly-P bacteria into wastewater for landfill leachate treatment has been found to be a cost-effective practive, a viable technology in terms of environmental protection and sustainability, especially in the developing-countries. For optimum struvite crystallization from landfill leachate, the Mg:PO_4 molar ratio as (1.2:1) was used, the pH of reaction was adjusted to 9 and the sample was stirred continously during 40 minutes. The supernatant sample was then added 1% nitrogen removal bacteria (Pseudomonas stutzeri D3b strain) and 1% poly-P bacteria (Kurthia sp. TGT1013L strain), 5 g glucose/L and aeration 12/24h during 3 days, ammonium concentration reduced significantly from 1076 mg/L to 1.5 mg/L and orthophosphate concentration decreased noticeably from 24.91 mg/L to 7.6 mg/L.*

***Keywords*— ammonium, bacteria, landfill leachate, orthophosphate, pH, struvite precipitation.**

I. INTRODUCTION

Wastewater is usually hazardous to human populations and the environment and must be treated prior to disposal into streams, lakes, seas, and land surfaces [1]. Obligatory anaerobic treatment of domestic and agro-industrial wastewater releases large amounts of phosphorus and nitrogen into wastewater. These nutrients are directly responsible for eutrophication (extraordinary growth of algae as a result of excess nutrients in water bodies) of rivers, lakes, and seas worldwide [2, 3]. Consequently, disposal of wastewaters produces a constant threat to dwindling fresh water on a global scale [4].

Landfill leachate treatment is an important issue of the waste management system in municipal areas [5, 6, 7]. Their quantity and quality depends on a number of factors: the type of deposited wastes as well as the age of the landfill and the phase of waste decomposition. The leachates withdrawn from landfill in methanogenic phase (methanogenic leachates) are characterized by high nitrogen load and large amount of refractory organic compounds with a high chemical oxygen demand/biochemical oxygen demand (COD/BOD) ratio [7]. Landfill leachate that is collected and removed from a landfill must be managed in a suitable manner. This involves some type of treatment process whether on or off-site. The various methods and technologies tested, applied and proposed for the treatment of landfill leachate range from the recirculation of leachate through the landfill to the more sophisticated combination of physical, chemical and biological processes [8, 9, 10, 11,12]. Magnesium ammonium phosphate (MAP) precipitation is a physical/chemical technique [7, 5, 13], which produces $MgNH_4PO_4.6H_2O$ by the precipitation of magnesium, ammonium and phosphate under alkaline conditions. This precipitation method is advantageous due to its ability to effectively precipitate NH_4-N from wastewaters forming easily settleable insoluble compounds, which have a potential as a binding material in cement [14].

$$Mg_2^{+} + NH_4^{+} + H_2PO_4 + 6H_2O \longrightarrow MgNH_4PO_4^{-} + 6H_2O + 2H_2$$

The most promising compound for recovery from wastewater plants is magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4.6H_2O$), commonly known as struvite, which precipitates spontaneously in some wastewater processes [15, 16, 17]. If formation and collection are controlled and cost-effective, struvite might have potential in the fertilizer market. Struvite precipitates spontaneously in wastewater treatment environments where high concentrations of soluble phosphorus and ammonium are present. Additional essential conditions are low concentration of suspended solids and pH above 7.5. Precipitation of struvite requires that its components are available simultaneously in the wastewater in the molecular ratio 1(Mg^{2+}):1(NH_4^{+}):1(PO_4^{3}). Normally, municipal wastewater and several other wastewaters tend to be rich in ammonium, but deficient in magnesium, so supplementation of magnesium is required, and this helps to increase solution pH [18, 19, 20, 21, 22] . Similarly, addition of magnesium chloride or bittern, a low-cost magnesium, forced precipitation of phosphorus and reduced the concentration of soluble phosphorus in swine waste within a 10-min reaction time [23, 24] . The pH can also be

elevated by adding NaOH, an expensive process [16], by air stripping [25], where aeration of wastewater removes CO_2 and pH increases in the process [26] or by ion using phosphate-selective sorbents [27] . This process was viewed as enhanced biological phosphorus removal (EBPR) in wastewater treatment systems [28, 29, 30].

This study investigates the following steps: (1) struvite pretreatment of raw landfill leachate, (2) applying nitrogen removal bacteria and poly-P bacteria to remove N and P out leachate. With The objectives of this study were: (1) to investigate the effects of pH and molar ratios for magnesium, and phosphate ions on ammonia N and phosphate P removal from raw landfill leachate, (2) to apply nitrogen removal bacteria and poly-P bacteria to enhance the waste disposal process.

II. MATERIAL AND METHOD

2.1 Raw landfill leachate

The composition of raw landfill leachate used in this study (collected from Kinh Cung landfill, Phung Hiep dist., Hau Giang province, Vietnam) was presented in Table 1.

TABLE 1
THE MAJOR COMPOSITION OF RAW PIGGERY WASTEWATER

Content	Concentration
pH	8.79
TSS (mg/L)	890 (very dark)
TN (mg/L)	392
TP (mg/L)	23.3
NH_4^+ (mg/L)	1076
PO_4^{3-} (mg/L)	24.9

2.2 Chemicals

Magnesium sulphate hexahydrate ($MgSO_4.7H_2O$) and calcium dihydrogen phosphate $Ca(H_2PO_4).2H_2O$ were employed as magnesium and phosphate sources, respectively. pH was adjusted using 10 and 6 M sodium hydroxide, as well as concentrated sulfuric acid (98%). All chemicals used in the study are analytical grade.

2.3 Struvite precipitation

The initial precipitation experiments for optimization were carried out in 1-L beakers. Precipitation was initiated with the addition of the desired amount of $MgSO_4.7H_2O$ and $Ca(H_2PO_4).2H_2O$ at stoichiometric ratios with pH adjusted by using NaOH. Mixing with the use of magnetic stirrer was continued until a steady pH. During MAP reaction, the pH of samples was adjusted to desired values by adding gradually 6N or 10N NaOH. Subsequent to this, the mixtures were allowed to settle for 30 min and the supernatant samples were collected for ammonium and orthophosphates analyses. Chemicals were added into landfill leachate to receive struvite, applying to equation as follows

$$m = (n - a) \times M$$

with:

- m is weigh of chemicals adding into landfill leachate ($MgSO_4.7H_2O$ and $Ca(H_2PO_4)2.H_2O$)
- n (nmol) of mol NH_4^+ in 1 litre landfill leachate
- a (nmol) of mol Mg^{2+} (or PO_4^{3-}) in 1 litre landfill leachate
- M is block molecule of $MgSO_4.7H_2O$ and $Ca(H_2PO_4)2.H_2O$

2.4 Effects of pH, ratio of mol Mg and PO4 and stirring time on struvite crystallization

Exp.1. The pH treatments were estimated at 8, 9, 10 while control was not adjusted pH. The Mg^{2+}:NH_4^+:PO_4^{3-} molar ratio was controlled at 1:1:1. Each treatment replicated 3 times and the volume of reaction was 0.5 L landfill leachate

Exp.2. Based on the result of preliminary test (Exp. 1), the subsequence was then carried out at the optimum pH. Five ratios of mol (Mg^{2+}:NH_4^+:PO_4^{3-}) ratio of 1:1:1, 1.2:1:1, 1.5:1:1, 1:1:1.2 and 1:1:1.5 were estimated with three replications, each replication was 1 beaker 1-L containing 0.5 L landfill leachate.

Exp.3. Optimal stiring time (0, 10, 20, 40, 60, 80, 100 and 120 min) was conducted with three replications, and each replication was a beaker 1-L containing 0.05 L landfill leachate.

The objective of these three experiments was not only the highest struvite precipitation but also removed ammonium and orthophosphate out of landfill leachate.

Landfill leachate samples after precipitation process above were filtered through filter paper (Ø11 cm, Hangzhou Spacial Paper Industry Co. Ltd, Zhejiand, China) and the supernatant samples were analysed ammonium and orthophosphate concentration at Advanced Lab., Can Tho University, Vietnam**.**

2.5 Application of nitrogen removal bacteria and poly-P bacteria in landfill leachate treatment

After experiment 3, the supernatants (of landfill leachate) were analysed ammonia and orthophosphate concentration, and they were then added nitrogen removal bacteria (*Pseudomonas stutzeri* D3b strain) [31] and poly-P bacteria (*Kurthia* sp. TGT013L strain) [32] (0.5%), glucose (5 g/L), aerotion with different times (6, 12, 18 and 24/24 h). The experiment was completely randomized design with 3 replications, the experiment constited of 6 treatments as follows:

1. T1: control [without bacteria and aeration],
2. T2: landfill leachate [after withdrawal struvite] without bacteria and aeration,
3. T3: T2 applied D3b + TGT13L + aeration 6/24h
4. T4: T2 applied D3b + TGT13L + aeration 12/24h
5. T5: T2 applied D3b + TGT13L + aeration 18/24h
6. T6: T2 applied D3b + TGT13L + aeration 24/24h

Each treatment was one 2-L plastic containter containing 1L landfill leachate

The result from the above experiment was done with 10-L bigger plastic container containing 5 litres landfill leachate and the experiment with only two treatments as control and optimal treatment were conducted with 3 replications.

2.6 Analytical methods

NH_4^+-N (Colometric method or Phenol nitroprusside method) [33], COD, BOD, Orthophosphate (Colormetric method) and pH (pH meter) were determined by Advanced Analyses Laboratory, Can Tho University, Viet Nam.

III. RESULTS AND DISCUSSIONS

3.1 Effect of pH on NH_4^+-N and PO_4_P removal during struvite precipitation

In table 2 showed that the optimization of struvite precipitation for pretreatment of the raw landfill leachate was pH 9 (21.17 g/L) and at pH 9 obtained at the theoretical stoichiometric ratio of Mg^{2+}:NH_4^+:PO_4^{3-} ratio of 1.2:1:1 the highest struvite precititation (26.34 g/L).

TABLE 2
EFFECTS OF PH AND Mg:PO_4 ON AMOUNT OF STRUVITE CRYSTALLIZATION

Treatment	g/L	Treatment	g/L
Control*	12.43	1.0 mol Mg : 1 mol PO_4	22.52
pH=8	20.96	1.2 mol Mg : 1 mol PO_4	**26.34**
pH=9	**21.17**	1.5 mol Mg : 1 mol PO_4	21.30
pH=10	20.02	1 mol Mg : 1.2 mol PO_4	22.45
LSD.01	**1.49**	1.5 mol Mg : 1 mol PO_4	26.29
C.V (%)	**2.92**	**LSD.01**	**0.98**
		C.V (%)	**1.20**

After 40 minute stiring, 1.080 g struvite was formed from 50 ml landfill leacheate and this stirring time was the best in comparison with others or the optimal stirring time for struvie formation even through there was no difference between eight treatment significantly (Figure 1). The combination between ($MgSO_4.7H_2O$) and $Ca(H_2PO_4).2H_2O$ with NH_4 in landfill leachate happened slowly from 2 minute to 40 minute furthermore at 40 minute, ammonium concentration reduced lowest (Figure 4).

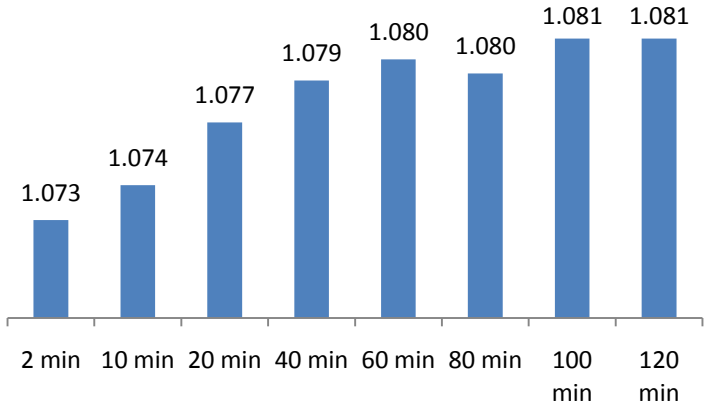


FIGURE 1. Effects of stirring time (min) on struvite formation (g/50 mL landfill leacheate)

Over 70.0% of ammonium and orthophosphate were removed at pH 9, with a residual concentration of 278 mg/L NH_4-N and 5.96 mg/L PO_4_P as depicted in Figure 2, thus indicated that pH 9 was the most suitable for struvite formation for the raw landfill leachate under investigation.

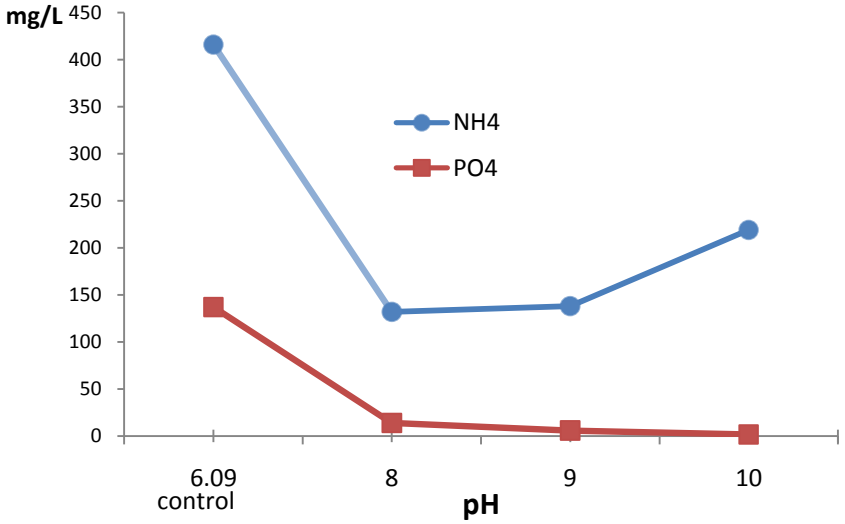


FIGURE 2. Effect of pH on NH_4^+-N and PO_4^{3-} removal during struvite formation

Similarly, molar ratio Mg:PO4 (1:1.2) was the best mol molecule to remove ammonium and orthophosphate in landfill leachate (Fugure 3).

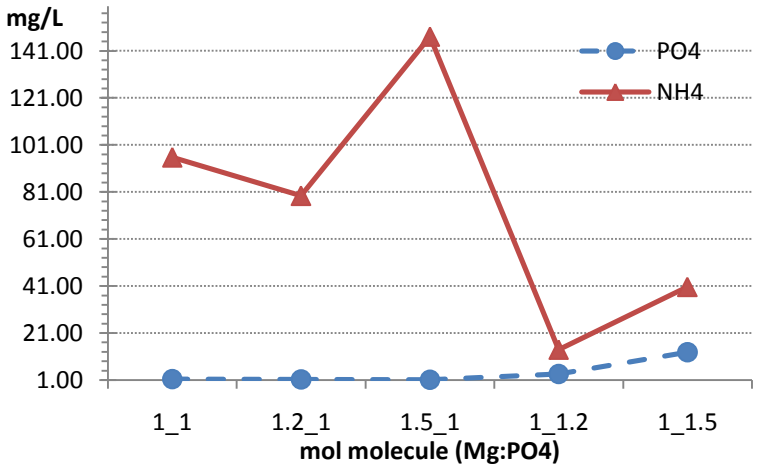


FIGURE 3. Effect of mol Mg:PO_4 on NH_4^+-N and PO_4^{3-} removal during struvite formation

From the above results (Table 2, Figure 2, Figure 3), pH 9, mol Mg:PO_4 (1:1.2:1) were chosen the optimal conditions for sturvite formation and the lowest ammonium and orthophosphate in landfill leachate.

In experiment 3, the landfill leachate was adjusted at pH 9 and mol Mg:PO_4 (1:1.2:1), the stiring time or aeration began, the result from Figure 4 showed that ammonium concentration reduced to the time but orthophosphate concentration in landfill leachate reduced to 40 minute and after that orthophsphate concentration increased to 120 minute, however struvite formation at all the times was not difference (Figure 1).

3.2 Effects of nitrogen removal bacteria and poly-P bacteria in landfill leachate treatment

In experiment 1-L, application of nitrogen removal bacteria (D3b strain) and poly-P bacteria (TGT013L strain) into landfill leachate reduced ammonium concentration to the time however aeration 12/24 h (NT4) reduced ammonium concentration at day 3 and saved energy (only aeration 12/24 h compared to 18/24 or 24/24 h).

Similarly, orthophosphate concentration in landfill leachate induced from day 1 to day 4 after that PO_4 concentration reduced perhaps aeration increased PO_4 level in landfill leachate, and PO_4 concentration of treatment 3 (NT3) decreased at day 5, day 6. Herewith day 3 reduced ammonium concentration (Figure 5), day 3 was chosen to low PO_4 concentration (Figure 6) for experiment 5L.

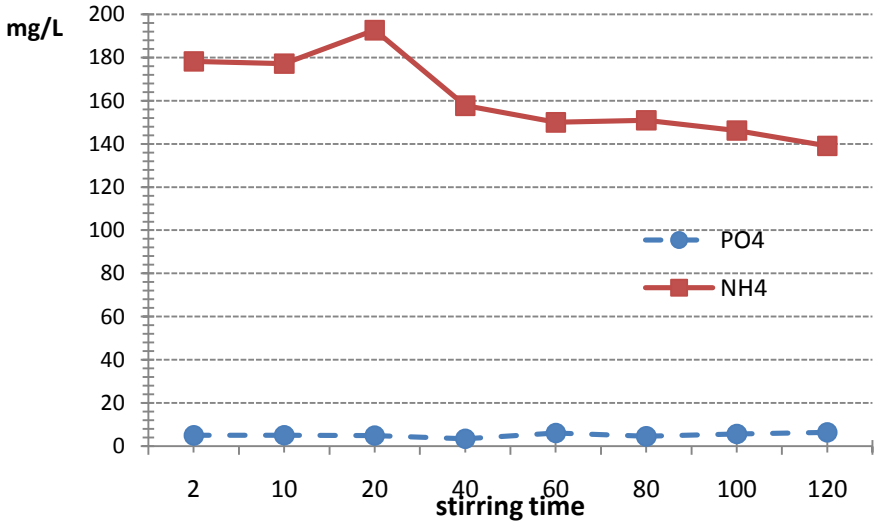


FIGURE 4. Effect of stirring time on NH_4^+-N and PO_4^{3-} removal concentration

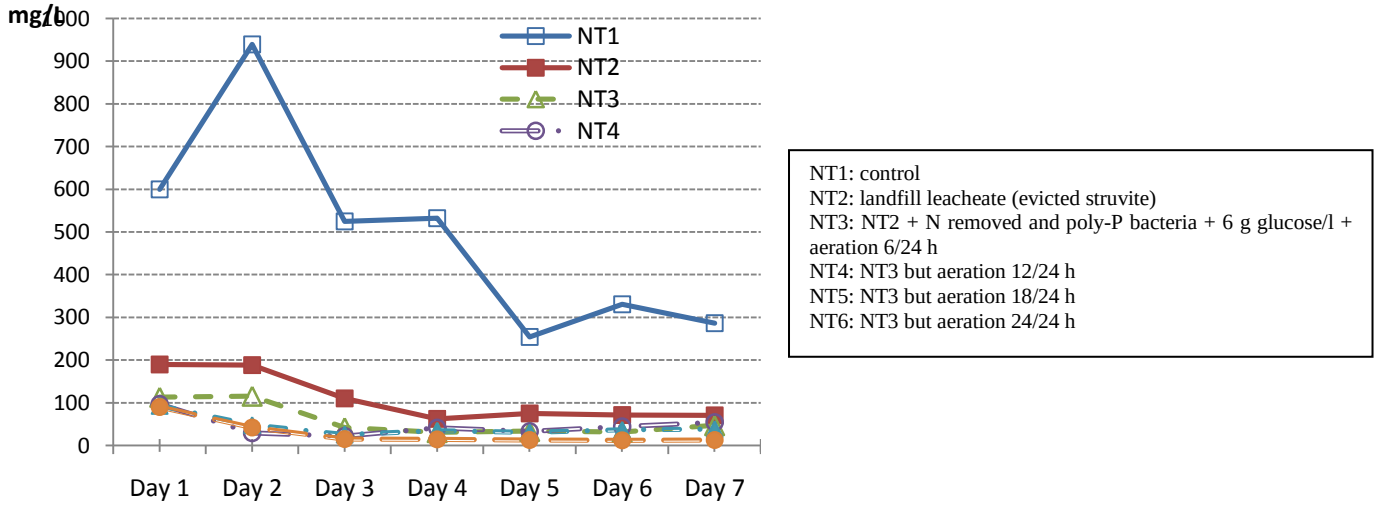


FIGURE 5. Effects of nitrogen removal bacteria and poly-P bacteria on ammonium concentration in landfill leachate

From the above results, in the experiment 5L, the experiment only two treatments: control (landfill leachate) without treatment and landfill leacheate applied Mg:PO_4 (1:1.2:1), pH 9, and stiring time in 40 minutes. Supernatant was appplied nitrogen removal bacteria and poly-P bacteria plus 5 glucose/L and aeration 12/24h in 3 days and the results as follows: 362.6 mg/L ammonia reduced to 1.5 mg/L (reached to A table, QC40:2011/BTNMT) and 9.6 mg/L PO_4^{3-} reduced to 7.6 mg/L (B table is 6 mg/L) however wastewater was irrigated in pond with *Lemma* sp., after 3 days ammonium and orthophosphate concentration in landfill leachate disappeared.

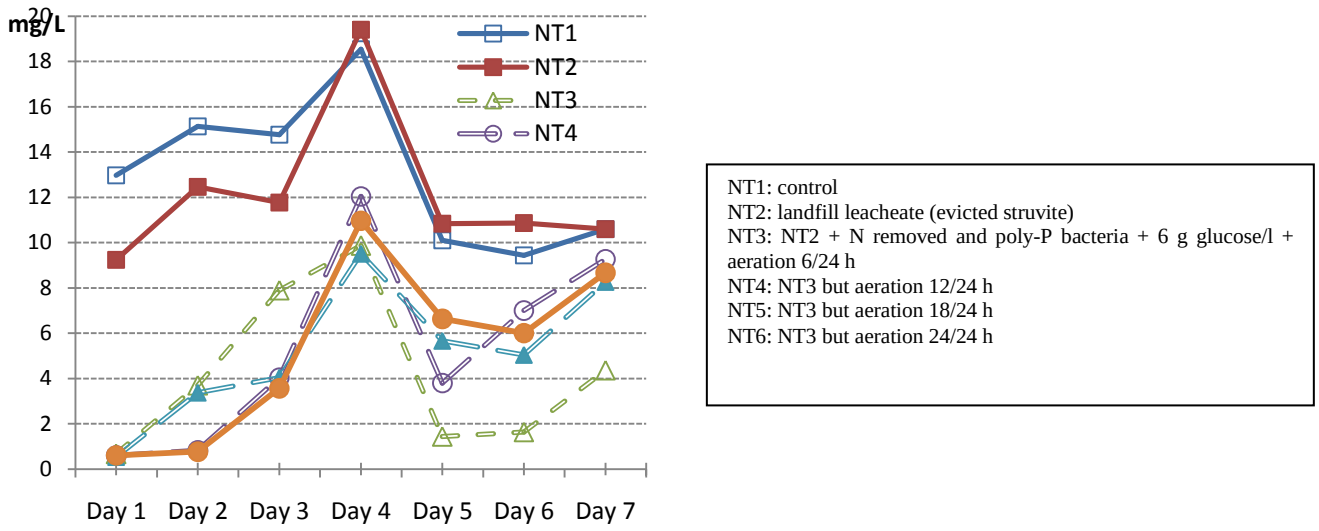


FIGURE 6. Effects of nitrogen removal bacteria and poly-P bacteria on orthophosphate concentration in landfill leachate

From the above results, in the experiment 5L, the experiment only two treatments: control (landfill leachate) without treatment and landfill leacheate applied Mg:PO_4 (1:1.2:1), pH 9, and stiring time in 40 minutes. Supernatant was appplied nitrogen removal bacteria and poly-P bacteria plus 5 glucose/L and aeration 12/24h in 3 days and the results as follows: 362.6 mg/L ammonia reduced to 1.5 mg/L (reached to A table, QC40:2011/BTNMT) and 9.6 mg/L PO_4^{3-} reduced to 7.6 mg/L (B table is 6 mg/L) however wastewater was irrigated in pond with *Lemma* sp., after 3 days ammonium and orthophosphate concentration in landfill leachate disappeared.

The pH plays an important role during the struvite precipitation process. Struvite or MAP can be precipitated at a wide range of pH (7.0–11.5), but the suitable pH ranges between 7.5 to 9.0 [34]. Efficiency of MAP precipitation depends on the concentration and molar ratios of $Mg_2^{+,}$ NH_+^4 , & PO_4^{3-} , pH, aeration rate, temperature, and presence of Ca^{2+} in the reacting media [35][34][16][36]. It is found that a wide range of PO_4 and Mg ratio was applied for struvite precipitation, but in most cases, the effective ratio was 1:1 or 1:1.2 (Rahman *et al*., 2011)[37]. The addition of chemicals to the wastewaters would be needed to provide an equimolecular condition of PO_4 and Mg. Yetilmezsoy and Zengin [35] conducted a series of experiments to see the effect of Mg, NH_4 and PO_4 ratio on struvite precipitation and nitrogen removal efficiency.

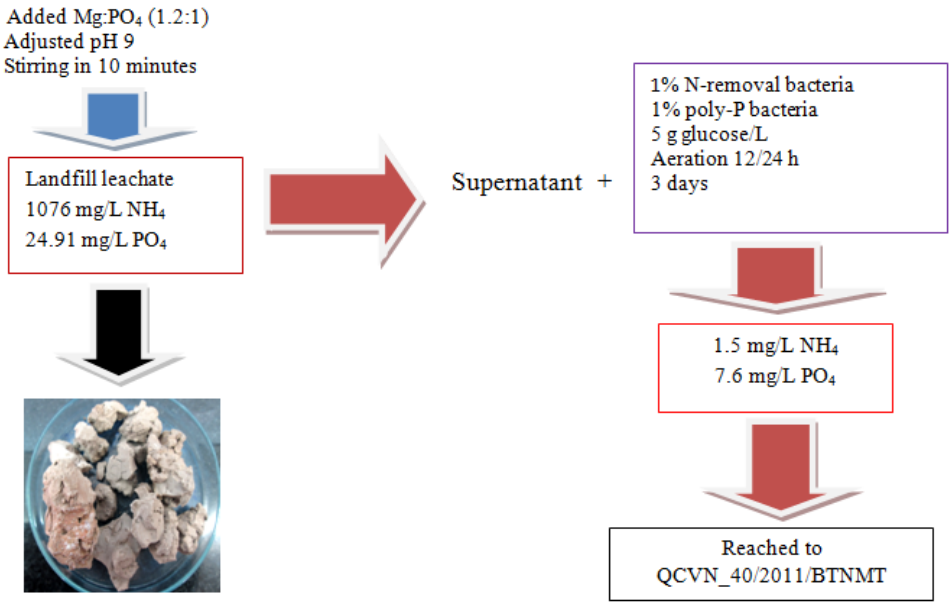
Yetilmezsoy and Zengin [35] stated that a sufficient aeration time should be provided to achieve high removal efficiencies. They obtained about 93.4% NH_4-N removal with an aeration rate of 0.6 L min^{-1} within a period of 24 h. They also found the highest NH_4-N removal (95.3%) in 12 h reaction time with an aeration rate of 10 L min^{-1}. Lei *et al*. [38]found about 60.2% ammonia removal with an aeration rate of 0.6 L min 1 in a reaction time of four hours. On the contrary, they achieved the same removal efficiency without aeration in a period of 24 h. Liu *et al*. [39][40]found that struvite formation is proportional to the aeration rate and reached a plateau at around 0.73 L min^{-1}. *Pseudomonas stutzeri* strain D3b was isolated from in wastewater of catfish fish-ponds in the Mekong Delta and its application for wastewater treatement effectively [31]. Application of *Pseudomonas stutzeri* D3b strain and *Acinetobacter lwoffii* TN7 strain to remove ammonia in wastewater of biowaste was carried out to evaluate their ability of ammonia removal at different concentrations with and without aeration condition in laboratory; The results showed that these species had ammonia removal ability effectively at both 50 mg/l and 100 mg/l ammonia. *Pseudomonas stutzeri* strain D3b and *Acinetobacter lwoffii* strain TN7 are the best bacterial species to remove ammonia. Besides that, both of species removed ammonia in aerobic condition better than anaerobic condition. In three days, the ammonia removal efficiency of *Pseudomonas stutzeri* D3b were 97.2% and 98.57% and *Acinetobacter lwoffii* TN7 were 96.32% and 98.31% in 50 mg/l and 100 mg/l ammonia concentrations in wastewater of biowaste, respectively

[41]. Polyphosphate accumulating organisms (PAOs) is known as the microorganisms to absorb free phosphate in the environment and assimilate them as intracellular polyphosphate (poly-P) particles. This process was viewed as enhanced biological phosphorus removal (EBPR) in wastewater treatment systems [28, 29, 30]. Khoi *et al*. [32] applied *Kurthia* sp. TGT013L to remove orthophosphate in wastewater effectively.

IV. CONCLUSION

Production of struvite from wastewaters will reduce the hazard of eutrophication in the water bodies by removing N and P. Production of struvite from wastewater and its utilization as fertilizer would partially help to reduce global warming and thus, it would be an effective eco-friendly fertilizer.

Treatment of piggery wastewater consisting of struvite eviction and removal of nitrogen and phosphate using nitrogen removal bacteria and poly-P bacteria were high effectiveness and low cost with process as follows:



REFERENCES

[1] L.E. de-Bashan, and Y. Bashan. "Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003)". Water Res. 2004, 38: 4222–4246.

[2] P.S. Lau, N.F.Y. Tam, and Y.S. Wong. "Wastewater nutrients (N and P) removal by carrageenan and alginate immobilized *Chlorella vulgaris*." Environ. Technol. 1997, 18: 945–951.

[3] C. Trepanier, S. Parent, Y. Comeau, and J. Bouvrette. "Phosphorus budget as a water quality management tool for closed aquatic mesocosms." Water Res. 2002, 36: 1007–1017.

[4] F. Montaigne, and P. Essick. "Water Pressure." Natl. Geog. 2002, 202: 2–33.

[5] S. He, Y., Zhang, M. Yang, W. Du, and H. Harada. "Repeated use of MAP decomposition residues for the removal of high ammonium concentration from landfill leachate." Chemosphere, 2007, 66: 2233–2238.

[6] I. Kabdaşli, I., A. Şafak, and O. Tünay O. "Bench-scale evaluation of treatment schemes incorporating struvite precipitation for young landfill leachate." Waste Manag. 2008, 28: 2386–2392.

[7] D. Kim, H.D. Ryu, M.S. Kim, J. Kim, and S.I. Lee. "Enhancing struvite precipitation potential for ammonia nitrogen removal in municipal landfill leachate." J Hazard Mater. 2007, 146: 81–85.

[8] S. Chen, S., D. Sun, and G.S. Chung. "Simultaneous removal of COD and ammonium from landfill leachate using an anaerobic–aerobic moving-bed biofilm reactor system." Waste Manage 2008, 28: 339–346.

[9] A. Altin. "An alternative type of photoelectro-fenton process for the treatment of landfill leachate." Sep Purif Technol. 2008, 61(3): 391–397.

[10] A.S. Qazaq, T. Hudaya, I.A.L. Lee, A. Sulidis, and A.A. Adesina. "Photoremediation of natural leachate from a municipal solid waste site in a pilot-scale bubble column reactor." Catal Commun. 2007, 8: 1917–1922.

[11] T. Robinson. "Membrane bioreactors: nanotechnology improves landfill leachate quality." Filtr. Sep. 2007, 44:38–39.

[12] T.A. Kurniawan, and G.Y.S. Chan. "Physico-chemical treatments for removal of recalcitrant contaminants from landfill leachate." J. Hazard Mater. 2006, B129: 80–100.

[13] J.D. Doyle, and S.A. Parsons. "Struvite formation, control and recovery." Wat. Res. 2002, 36: 3925–3940.

[14] R.D. Schuiling, and A. Andrade. "Recovery of struvite from calf manure." Environ. Technol. 1999, 20:765–768.

[15] N.A. Booker, A.J. Priestley, and I.H. Fraser. "Struvite formation in wastewater treatment plants: opportunities for nutrient recovery." Environ. Technol. 1999, 20: 777–782.

[16] I. Stratful, M. D. Scrimshaw, M. D., and J. N. Lester. "Conditions influencing the precipitation of magnesium ammonium phosphate." Water Res. 2001, 35: 4191–4199.

[17] S. Williams. "Struvite precipitation in the sludge stream at slough wastewater treatment plant and opportunities for phosphorus recovery." Environ. Technol. 1999, 20:743–747.

[18] N. Lee, P.H. Nielsen, H. Aspegren, M, Henze, M, K.H. Schleifer, and J.L. Jansen. "Long-term population dynamics and in situ physiology in activated sludge systems with enhanced biological phosphorus removal operated with and without nitrogen removal." Syst. Appl. Microbiol. 2003a, 26: 211–227.

[19] J.M. Chimenos, A.I. Fernandez, G. Villalba, M. Segarra, A. Urruticoechea, B. Artaza, and F. Espiell. "Removal of ammonium and phosphates from wastewater resulting." Water Res. 2003, 37: 1601–1607.

[20] E.V. Munch, and K. Barr. "Controlled struvite crystallization for removing phosphorus from anaerobic digester side-streams." Water Res. 2001, 35: 151–159.

[21] N.O. Nelson, R.L. Mikkelsen, and D.L. Hesterberg. "Struvite precipitation in anaerobic swine lagoon liquid: effect of pH and Mg: P ratio and determination of rate constant." Bioresource Technol. 2003, 89: 229–236.

[22] P. van Rensburg, E.V. Musvoto, M.C. Wentzel, and G.A Ekama. "Modelling multiple mineral precipitation in anaerobic digester liquor." Water Res. 2003, 37: 3087–3097.

[23] R.T. Burns, L.B. Moody, I. Celen, and J.R. Buchanan. "Optimization of phosphorus precipitation from swine manure slurries to enhance recovery". Water Sci. Technol. 2003, 48: 139–146.

[24] S.I. Lee, S.Y. Weon, C.W. Lee, and B. Koopman. "Removal of nitrogen and phosphate from wastewater by addition of bittern." Chemosphere. 2003b, 51: 265–271

[25] S. Kalyuzhnyi, V. Sklyar, A. Epov, I. Arkhipchenko, I. Barboulina, O. Orlova, A. Kovalev, A. Nozhevnikova, and K. Klapwijk. "Sustainable treatment and reuse of diluted pig manure streams in Russia—from laboratory trials to full-scale implementation." Appl. Biochem. Biotech. 2003, 109: 77–94.

[26] P. Battistoni, G. Fava, P, Pavan, A. Musacco, and F. Cecchi. Phosphate removal in anaerobic liquors by struvite crystallization without addition of chemicals: preliminary results. Water Res. 1997, 31: 2925–2929.

[27] D. Petruzzelli, L. De Florio, A. Dell'Erba, and L. Liberti. "A new phosphate-selective sorbent for the Rem Nut (R) process. Laboratory investigation and field experience at a medium size wastewater treatment plant." Water Sci. Technol. 2003, 48: 179–184.

[28] P.L. Bond, R. Erhart, M. Wagner, J. Keller, and L. L. Blackall. "Identification of some of the the major groups of bacteria in efficient anf non-efficient biolical phosphorus removal activated sludge systems". Appl. Environ. Microbiol., 1999, 65(9): 4077-4088.

[29] T. Mino, M.C.M.Van Loosdrecht and J. J. Heijnen. "Microbiology and biochemistry of the enhanced biological phosphate removal process". Water Res. 1998, 32: 3193–3207.

[30] A. Oehmen, P. C. Lemos, G. Carvalho, Z. Yuan, J. Keller, L. L. Backall, and M.A.M.Reis. "Advances in enhanced biological phosphorus removal: from micro to macro scale". Water Research. 2007, 41: 2272-2300.

[31] C.N. Diep, C. N., P. M. Cam, N. H. Vung, T. T. Lai and N. T. Xuan My. "Isolation of *Pseudomonas stutzeri* in wastewater of catfish fish-ponds in the Mekong Delta and its application for wastewater treatment". Bioresource Technology, 2009, 100: 3787-3791.

[32] L.Q. Khoi, and C. N. Diep. "Isolation and phylogenetic analysis of polyphosphate accumulating organisms in water and sludge of intensive catfish ponds in the Mekong Delta, Vietnam." *American Journal of Life Sciences.* Vol. 1, No. 2, 2013, 61-71. doi: 10.11648/j.ajls.20130102.17 (online). ISSN 2328 - 5737

[33] D.R.Keeney, and D. W. Nelson. "Nitrogen-inorganic forms." In: Page, A.L.R.H. Miller and D.R. Keeney (eds.) Methods of soil analysis. Part 2-Chemical and microbiological properties. (2nd Ed.). Agronomy 9, 1982, pp.643-698.

[34] X.D. Hao, C.C. Wang, L. Lan, and M.C.M. Von Loosdrecht, "Struvite formation, analytical methods and effects of pH and Ca^{2+}." Water Sci. Technol. Vol. 58, 2008, pp.1687–1692.

[35] K. Yetilmezsoy, and Z.S., Zengin, "Recovery of ammonium nitrogen from the effluent of UASB treating poultry manure wastewater by MAP precipitation as a slow release fertilizer." J. Hazard. Mater. Vol. 166, 2009, pp. 260–269.

[36] I. Stratful, M, Scrimshaw, and J. Lester, "Conditions influencing the precipitation of magnesium ammonium phosphate". Water Res. Vol. 35, 2001, pp.4191–4199.

[37] K.S. Le Corre, E. Valsami-Jones, P. Hobbs, and S.A. Parsons, "Impact of calcium on struvite crystal size, shape and purity". J. Cryst. Growth. Vol. 283, 2005, pp. 514–522.

[38] M.M. Rahman, Y.H. Liu, J.H. Kwag, and C.S. Ra, "Recovery of struvite from animal wastewater and its nutrient leaching loss in soil". J. Hazard. Mater. Vol. 186, 2011, pp.2026–2030.

[39] X. Lei, S. Shimada, K. Intabon, and T. Maekawa, "Pretreatment of methane fermentation effluent by physico-chemical processes before applied to soil trench system." Agric. Eng. Int.: CIGR E J. vol. 8, 2006, 1–15.

[40] Y.H. Liu, M.M, Rahman, J.H., Kwag, J.H., Kim, and C.S. Ra, "Eco-friendly production of maize using struvite recovered from swine wastewater as a sustainable fertilizer source." Asian-Aust. J. Anim. Sci. vol. 24, 2011b. pp.1699–1705.

[41] Y.H. Liu, S. Kumar, J.H. Kwag, J.H., Kim, J.D.Kim, and C.S Ra, "Recycle of electrolytically dissolved struvite as an alternative to enhance phosphate and nitrogen recovery from swine wastewater." J. Hazard. Mater. Vol. 195, 2011c, pp.175–181.

[42] C.N. Diep, và N. T. H. Nam. 2012. Ứng dụng vi khuẩn *Pseudomonas stutzeri* và *Acinetobacter lwoffii* loại bỏ amoni trong nước thải từ rác hữu cơ. Tạp chí Khoa học Trường Đại học Cần Thơ 22b: 1-8. (Vietnamese).

Effects of Preservation using Response Surface Methodology on Fresh-cut Taro

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***Abstract*—** *In order to evaluate the effect of preservation on fresh-cut taro, the edible coatings preservatives were connected with Konjac glucan-mannan (KGM), potassium sorbate, chitosan, by determining changes of weight loss rate, browning degree (BD), polyphenol oxidase (PPO) and peroxidase (POD), the preservative effect on fresh-cut taro were studied. The optimal composite of Konjac glucan-mannan (KGM), potassium sorbate, chitosan was analyzed by single factor test and Box-Behnken response surface method. The results showed the optimal concentration were KGM 6g/L, potassium sorbate 4g/L and chitosan 8g/L. Verified test showed that composite coating could reduce the weight loss rate, browning, PPO and POD activity of fresh-cut taro.*

***Keywords*— fresh-cut taro; browning degree; response surface; Konjac glucan-mannan (KGM).**

I. INTRODUCTION

Colocasia esculenta (L), also known as taro, its edible part is the main underground bulb. Like other fresh-cut products, fresh-cut taro is also very easy to brown during processing and storage, which seriously affects the commercial value of the product. Konjac glucomannan is a natural plant polysaccharide, which can be dissolved in water will form a highly viscous pseudoplastic solution. At present, the research on konjac glucomannan film coating in fresh fruits and vegetables has been reported, but composite coating preservation of konjac glucomannan on taro has not been reported. Chitosan is a polysaccharide biological macromolecule, which has the advantages of safe, non-toxic, film-forming and bacteriostatic, edible and biodegradable, and has been widely reported [1] in recent years. The preservative potassium sorbate used in food was used as strengthening agent [2]. The best combination of Konjac glucomannan, potassium sorbate and chitosan was screened by single factor experiment and response surface design-expert analysis, exploring its mechanism of action, And providing technical support and theoretical basis for the application of konjac glucomannan composite coating film in taro storage and preservation.

II. MATERIAL AND METHOD

2.1 Materials and Reagents

2.1.1 Main raw material

Taking individual integrity, normal color, no damage longxiang taro, bought in taizhou.

Konjac glucomannan (KGM) : hefei bomei biotechnology co., LTD. Potassium sorbate: food grade, wang long group co. LTD. Chitosan: food grade, yunsheng biotechnology co. LTD.

2.1.2 Main instruments and equipment

GYB series high pressure homogenizer: Shanghai donghua high pressure homogenous machine factory; Slicer: yantai haidu food machinery co., LTD. Knives, stainless steel basins, preservation cabinets, etc.

2.2 Experimental methods

2.2.1 The single anti-browning agent used to inhibit the browning of taro

After the skin was peeled, it was cut into thin slices of 3~4 mm thick by slicing machine and randomly divided into 4 groups, respectively using distilled water (control) and KGM (concentration of 2, 4, 6, 8, 10.0 g/L respectively), edible chitosan(concentration of 6, 8, 10, 12, 14 g/L respectively) and potassium sorbate (concentration of 2, 4, 6, 8, 10.0 g/L respectively) solution for 5 min, after natural air at room temperature, placed in 4℃fresh-keeping cabinet storage, every 2 d to determine indicators.

Based on single factor experiment, three anti-browning agents KGM, chitosan and potassium sorbate were optimized by using the box-Behnken center combined experimental design. The experimental factors and horizontal design are shown in table 1[3].

TABLE 1
THE OPTIMIZATION FACTORS AND LEVEL OF RESPONSE SURFACE

level	factors		
	A:KGM concentration (g/L)	B:chitosan concentration (g/L)	C:Potassium sorbate (g/L)
1	4	8	4
2	6	10	6
3	8	12	8

2.2.2 Indicators Determination

Browning degree: With reference to the method of the Su xin guo[4], represented by OD_{410}×100;

Activity of polyphenol oxidase (PPO): by using catechol method [5], an enzyme activity unit (U) was increased by 0.01 in OD_{398} 1min;

Activity of Peroxidase (POD) : the use of guaiacol[6] increased by 0.01 in OD_{470} 1min to 1 enzyme activity unit (U).

The above indexes were repeated 3 times

III. RESULTS AND ANALYSIS

3.1 Single factor experiment

3.1.1 Effect of chitosan on the browning of fresh-cut taro

As can be seen from figure 1, the browning degree of fresh-cut taro was increasing continuously during storage. In the control group, the Browning of the 1d was increased, until 6d, the tangent plane was severely browned, and the browning degree reached 9.2, which was 3.47 times higher than the beginning and lost the quality of food. The browning degree of the fresh-cut taro treated with chitosan coating was significantly less than that of the control group ($p<0.05$), and the group of 6 g/L chitosan also reached 8.7 in the 8d. After comprehensive consideration, the optimal chitosan concentration was selected 12 g/L.

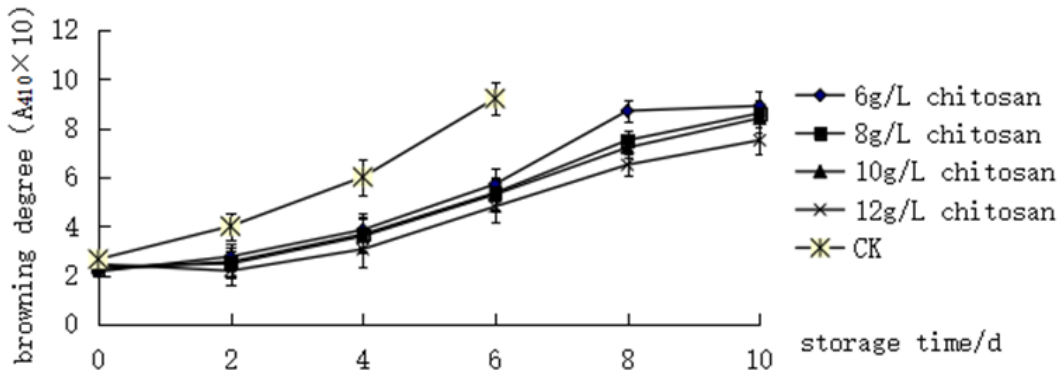


FIGURE 1: EFFECT OF CHITOSAN ON THE PRESERVATION

3.1.2 Effect of potassium sorbate on the browning of fresh-cut taro.

As can be seen from figure 2, the browning degree was increased gradually with the extension of storage time. The browning degree of fresh-cut taro treated with potassium sorbate was significantly less than that of control. In 4, 6, 8, 10 g/L potassium sorbate treatment group can be better inhibiting browning of taro, which may be the potassium sorbate as anti browning agents interfere with enzymatic browning reaction [7], eventually led to the effect of inhibiting browning. The group of 10 g/L sorbate treatment was not significantly different from the other 3 groups ($p>0.05$). In terms of economy, the group of 8 g/L was selected as the optimum level for composite coating composite response surface

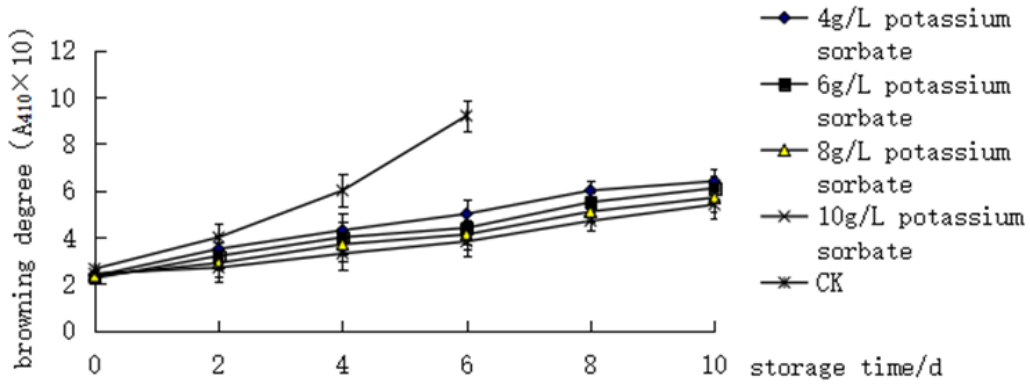


FIGURE 2: EFFECT OF POTASSIUM SORBATE ON THE PRESERVATION

3.1.3 Effect of KGM on browning of fresh-cut taro

As can be seen from figure 3, compared with the treatment group, the browning degree of the control group was significantly increased, and the browning had already occurred in the storage of 1d. The browning degree of KGM was slowly rising. It may be that KGM deals with a thin film on the surface of the taro to isolate the contact between polyphenol oxidase and O_2[8,9]. Among them, the group of 6 g/L had better preservation effect than other groups.

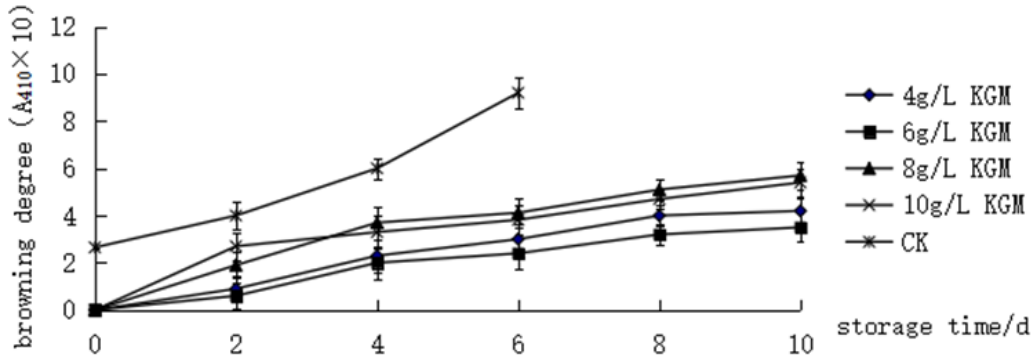


FIGURE 3: EFFECT OF KGM ON THE PRESERVATION

3.2 The response surface test of optimized composite combination

Based on the single factor, the optimization composition of preservation process was carried out, and the results of response surface test were shown in table 2, and the regression analysis was shown in table 3.

TABLE 2

THE OPTIMAL COMPOSITION OF PRESERVATION TESTED IN RESPONSE SURFACE ANALYSIS

Test	A KGM concentration (g/L)	B chitosan concentration (g/L)	C Potassium sorbate (g/L)	Browning degree
1	0(6)	1	-1(4)	4.13
2	1(8)	-1(8)	0(6)	4.25
3	0	-1	-1	2.04
4	-1(8)	1(12)	0	4.87
5	0	0(10)	0	1.15
6	0	0	0	1.17
7	0	0	0	1.11
8	-1	0	-1	1.67
9	1	1	0	4.05
10	0	0	0	1.19
11	-1	-1	0	3.17
12	1	0	-1	6.00
13	-1	0	1(8)	4.28
14	0	-1	1	3.05
15	0	1	1	6.87
16	0	0	0	1.16
17	1	0	1	6.47

Table 2 can be obtained after regression fitting:

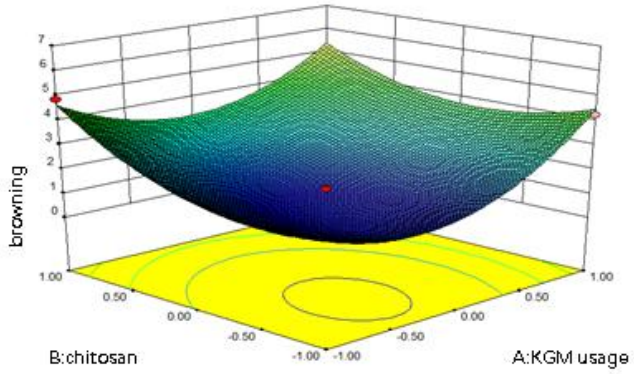
$Y=5.15+0.76A+1.59B+0.85C-0.30AB-0.54AC+0.43BC-0.90A^2-1.49B^2+0.36C^2$.

TABLE 3
EXPERIMENTAL DESIGN AND RESULTS FOR RESPONSE SURFACE ANALYSIS

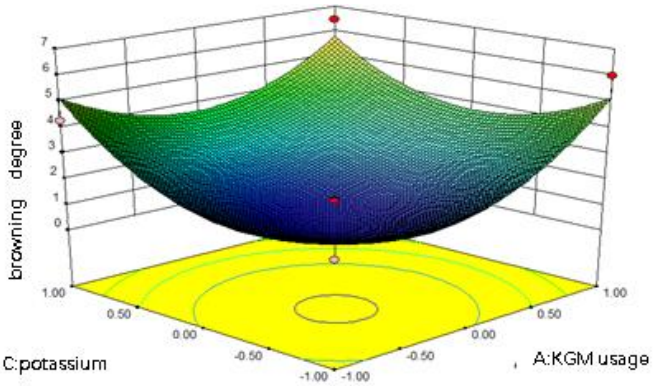
Project	sum of squares	freedom	mean square	F value	P value	significant
model	46.49	9.00	5.17	5.82	0.015	*
A	4.62	1.00	4.62	5.21	＜0.0001	**
B	20.19	1.00	20.19	22.76	0.0020	**
C	5.83	1.00	5.83	6.57	0.03	**
AB	0.36	1.00	0.36	0.41	0.54	
AC	1.14	1.00	1.14	1.29	0.29	
BC	0.75	1.00	0.75	0.84	0.39	
A^2	0.19	1.00	0.19	0.27	0.04	*
B^2	9.30	1.00	9.30	10.48	0.01	*
C^2	9.19	1.00	9.19	12.79	＜0.0001	**
residual	5.03	7.00	0.72			
loss item	3.85	3.00	1.28	4.37	0.09	
net error	1.18	4	0.29			
Correction term	52.70	16				

Note:* the influence was significant, P<0.05; ** the influence was very significant, P < 0.01.

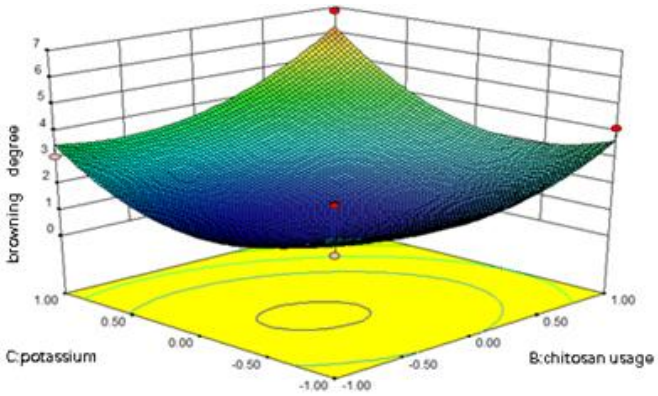
As can be seen from table 3, the model had a significant one, and the quadratic term A^2, B^2 and C^2 were significant. The F value of the model was 5.82, and the significant level of the overall model was P< 0.05, indicating that the model was significant. The model R^2 = 0.9647 indicates that the regression equation has a good fitting degree and a smaller loss, which can be used to substitute the real test point for analysis. According to the obtained mathematical model, the response surface diagram was drawn, as shown in figure 4.



A. THE INTERACTION OF CHITOSAN USAGE AND KGM USAGE



B. THE INTERACTION OF POTASSIUM SORBATE USAGE AND KGM USAGE



C. THE INTERACTION OF POTASSIUM SORBATE USAGE AND CHITOSAN USAGE

FIG.4 THE RESPONSE OF THE TWO INTERACTING FACTORS ON BROWNING DEGREE AND CONTOUR MAP

Potassium sorbate, chitosan and KGM interaction of taro had influence on browning degree of coating preservation, especially the dosage of KGM was obvious, which affected by the interaction of KGM and chitosan usage, in response to the surface map, which showed a large gradient, changed significantly (figure 4).

3.3 Optimization of Compound Preservative

After the analysis of the boundary value and the obtained extremum by the design-expert software, the optimal conditions for each factor obtained were 8 g/L of chitosan, 4.34 g/L of potassium sorbate, and KGM5.83 g/L, and the predicted browning value was 3.26 under this condition. Considering the actual operation, the above conditions were modified as: chitosan 8 g/L, potassium sorbate 4 g/L, KGM6 g/L.

The experimental results showed that the browning degree was 3.14 and the prediction accuracy was as high as 96.31% in the best condition of correction, and the accuracy of regression model was proved again.

3.4 The preservative effect of KGM composite coating on taro

3.4.1 Effect of KGM composite coating on the loss weight of fresh-cut taro

As can be seen from figure 5, the quality loss rate of the control group (CK) was increased rapidly during storage, and the change was positively correlated with the storage time.

The mass loss of control treatment and KGM composite coating on taro were 8.02%, 5.20% respectively when storage 6 d, which may be chitosan in composite KGM coating in fresh-cut taro surface film to prevent moisture evaporate[10]. It was proved that the treatment of KGM composite coating could effectively reduce the quality loss of taro.

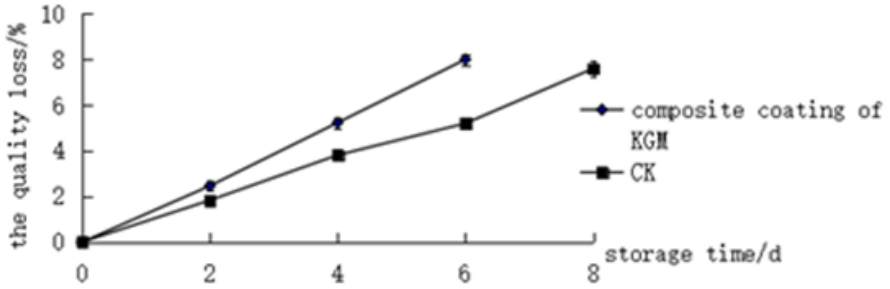


FIGURE 5: EFFECT OF KGM COMPOSITE COATING ON THE QUALITY LOSS OF FRESH-CUT TARO

3.4.2 Effect of KGM composite coating on PPO activity of fresh-cut taro

As can be seen from figure 6, the PPO activity of taro was rising continuously during storage. In contrast (CK), the growth rate of KGM composite coating was slow. In storage of 10d, the PPO activity of the coating group was 18.93 U/g than the control group (25.78 U/g), which may be due to the effect of moderate ascorbic acid in the PPO reaction system. It can be seen that composite coating of KGM could reduce the PPO activity that triggered the enzymatic reaction.

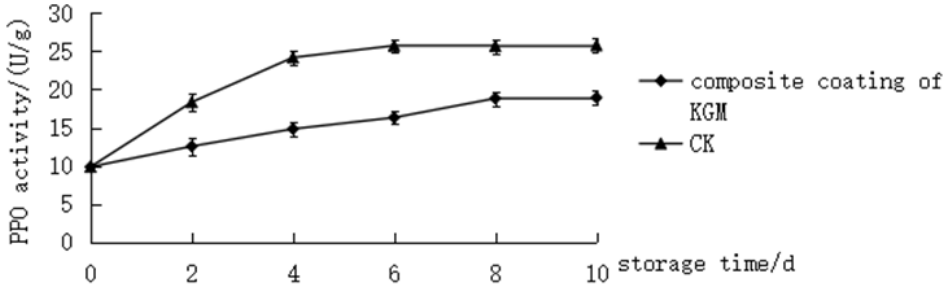


FIGURE 6: EFFECT OF KGM COMPOSITE COATING ON PPO ACTIVITY OF FRESH-CUT TARO

3.4.3 Effect of KGM composite coating on POD activity of fresh-cut taro

It can be seen from figure 7 that the POD activity of taro was increased with the increase of storage time. When the taro storage was 8d, the POD activity of control (CK) increased by 9.8 times, and the POD activity of KGM composite coating was 12.85u /g, which was 60.32% lower than the control group. It can be seen that KGM composite coating could effectively inhibit POD activity.

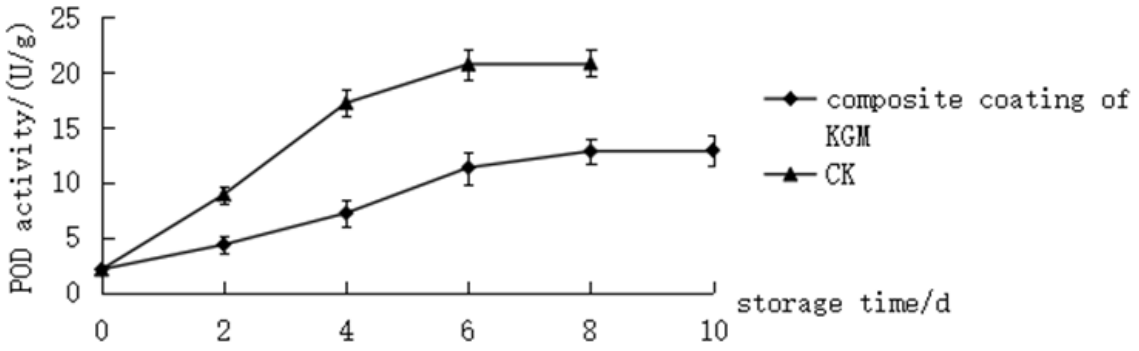


FIGURE 7: EFFECT OF KGM COMPOSITE COATING ON POD ACTIVITY OF FRESH-CUT TARO

IV. CONCLUSION

Through the quadratic polynomial regression equation model, the optimal proportion of KGM composite film was ethyl chitosan 8 g/L, potassium sorbate 4 g/L, KGM 6 g/L, composite coating treatment on taro could reduce the quality loss, slow browning, the inhibition of PPO and POD activity.

ACKNOWLEDGEMENTS

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REFERENCES

[1] HU Wei-rong, Liu Shun-zhi, Huang Hui-jua. Effects of Ascorbic Acid and Chitosan Treatments on Physiological Changes of Fresh-cut Taro during Storage. Food science,2010,31(24) : 436-439

[2] ZHOU Minshcng, Zhanu Guangjie, LI JunPing. Effect of Edible Film on Preservation of Strawberry During Storage. Northern Horticulture,2017,13) : 147-151

[3] ZHANG Yin. Effect of Konjac Glucomannan Composite Coating on Preservation of Blueberry. Jiangsu Agricultural Science,2017,45(11) : 146-149

[4] SUN Xinguo, Jiang Yueming, LI Yuebiao,etc. Effect of 4-HR on Browning and storage quality of fresh lotus root.food science,2003,24(12) : 142-145

[5] ZHANG Zhao-qi, Pang Xue-qun, DUAN Xue-wu,etc The Anthocyanin Degradation and Anthocyanase Activity During the Dericarp Browning of Lychee Fruit. China Agricultural Science, 2003,36(8) : 945-949

[6] LIU Shun-zhi, Ma Bin, Chen An-an,etc. Study on Fresh-keeping Effect of Chinese Herbal Medicine Extract on"Shatang"Mandarin.food science,2008,29(7) : 462-465

[7] XU Hai-xiang, Li Zhi-fang, Lu Yu-chen,etc. The Applied Study Retain Freshness on the Peeled Taro of Longxiang by Using Double Sodium Acetate. Food industry,2016,37(4) : 70-74

[8] MA Li-hua, QIN Wei-dong, CHEN Xue-hong,etc. Effect of Coating on Browning and Antioxidant Activity of Fresh-Cut Yam. Food science,2012,33(18) : 275-280

[9] WANG Qing, Sun Jin-yue, Liu Chao,etc. Study on Protein Extraction Technology from Peony Seed Meals Optimized by Response Surface Methodology. Food industry,2017,38(1) : 117-121

[10] Lin Jiao-fen, Li hui, Zhuang Yuan-hong,etc. Optimization of Recipe of Calcium-enriched Biscuit by Response Surface Methodology. Food industry,2017, 38(7) : 141-145.

Phosphotriesterase-Like Lactonase Immobilized on Zeolites for Pesticides Degradation

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Abstract— *In this study, a non commercial PTE was covalently immobilized on the NaX zeolite crystals and its ability to biodegrade the paraoxon to a less harmful compound was investigated. The immobilization method did not change the enzyme catalytic performance. In fact, the specific activity was the same of the free one. Besides, the process improved the stability of the enzyme.*

The obtained results are interesting because of usually the immobilization process increases the enzyme stability but causes a reduction of its catalytic activity. Therefore, this pioneer study of the immobilization of the PTE on zeolite particles seems to be an environmental-friendly solution to the problem of pesticides pollution.

Keywords— detoxification, enzyme immobilization, organophosphates, phosphotriesterase-like lactonase, specific activity, zeolite.

I. INTRODUCTION

Pesticides were and are extensively used in agriculture for controlling the spread of unwanted insects or weeds. The extensive use of pesticides causes serious environmental concerns; in fact, only 5% or less of the applied pesticides reach the target organisms which resulted in contamination of soil and water. Their continuous use brought an accumulation of pesticides and their residues in environment, endangering the populations by their varied toxicity [1]. Among the various groups of pesticides, organophosphates (OPs) are one of the most widely used types [2]. The current methods for the OPs compounds detoxification are harmful and determine serious environmental consequences. Therefore, the use of enzymes for the detection and decontamination of organophosphate agents has received considerable attention [2, 3]. Many enzymes catalyze the hydrolysis of the OPs among which the phosphotriesterases (PTE; EC 3.1.8.1) [4, 5] and PTE-like lactonases (PLL) [6]. Some of them are commercially available and were employed to purify OP-contaminated water. However, the main problem associated with the use of these enzymes is their low stability in solution [7].Usually, the enzymes are immobilized on inorganic or organic supports for improving their stability [8-10]. In addition, immobilization process facilitates the enzyme re-use and also leads to an easier separation of the enzyme from the reaction mixture, provides the control of the reaction time and reduces the enzyme loss in the product stream [11]. Among the various inorganic supports, zeolites have attracted significant attention for enzyme immobilization having remarkable properties: high surface area for high enzyme loadings, high chemical and mechanical stability [12]. Zeolites (alumino-silicate materials with well-defined pore size at molecular scale) are cost effective and non-toxic with respect to the health issues [13]. Considering these characteristics, they are suitable candidates for different applications such as water purification and softening, separation processes and sensors [14, 15] and for immobilization of enzymes [11, 16-18].

The topic of this work is the development of environmental-friendly materials for the treatment of waste water and for the remediation of contaminated sites. In particular, the study was focused on the covalent immobilization of a mutated version of the PLL from SsoPox namely *Sso*Pox W263F on the zeolite surface using the glutaraldehyde as cross linker. The immobilized enzyme was used for performing preliminary studies on the organophosphate pesticide (paraoxon ethyl) degradation.

II. MATERIAL AND METHOD

2.1 Materials

Glutaraldehyde (GLU), paraoxon ethyl and 4-nitrophenol were purchased from Sigma Adrich. Trizma Base ($C_4H_{11}NO_3$, ≥99.9%) and HCl, used for the preparation of TRIS/HCl buffer and HEPES sodium salt were also purchased from Sigma-Aldrich.

NaX zeolite particles (size ~ 2 μm; Sigma-Aldrich) were used as support for enzyme immobilization. Before the chemical modification, zeolite crystals were purified (*via* centrifugation) to enable the separation of the crystal fraction from the mother liquid. The solid phase was re-dispersed in distilled water and centrifuged again. The procedure was repeated for lowering the pH value from 10 to 7. Finally, the zeolite particles were heated at 500 °C for removing the amorphous organic materials and the water from the pores.

2.2 PTE production and purification

The enzyme SsoPox W263F, a mutated form of the wild type SsoPox from *Sulfolobus solfataricus*, was obtained, expressed in *E. coli* and characterized as previously reported [5]. The recombinant enzyme was produced in large scale (150 L) by coupling high cell density fermentation strategy with a galactose induction up to a 4660.0 $U \cdot L^{-1}$, as previously reported [19]. After extraction from the biomass by mechanical cell disruption, the enzyme was purified by coupling a thermo-precipitation step with a membrane-based ultra-filtration protocol. The final solution, containing the enzyme with a high purity grade, was freeze dried and preserved until it was used [19].

2.3 Zeolite crystals characterization

Morphology and size of NaX zeolite crystals, used as support for the enzyme immobilization, were analyzed by scanning electron microscopy (SEM) using a Cambridge Zeiss LEO 400 microscope. The Si/Al ratio of the zeolite crystals was determined by energy dispersive X-ray (EDX) performed with EDAX-Phoenix in SUTW Detector, analyzer: Si/Li crystal).

2.4 Chemical activation of zeolite crystals

The NaX zeolite activation was carried out by immerging 60 mg of crystals in a GLU solution (0.05M; pH= 5.0, buffer phosphate) for 24 hours at 45°C [8]. The modified zeolite particles were recovered by vacuum filtration and washed with buffer phosphate different times to remove the organic material adsorbed on its surface. The process was stopped when organic molecules (not covalently bound) were not detected in the washing solution by spectrophotometric analysis. Subsequently, the activated zeolite crystals were dried overnight at room temperature and used for immobilizing the enzyme by covalent binding, *via* cross-linking.

2.5 Enzyme immobilization

The enzyme was covalently immobilized (formation of a Schiff's base) by immerging the activated zeolite crystals (30 mg) in the enzyme solution (30 mL, 20 mM HEPES buffer pH 8.5) at 25 °C, under gentle stirring for 24 hours.

At the beginning, the effect of different concentrations of GLU (5 w/v %, 10 w/v %, 15 w/v %, 25 w/v %) on the amount of the immobilized enzyme was evaluated, keeping constant the initial enzyme concentration (2×10^{-3} $mg*mL^{-1}$). Afterwards, the best GLU concentration was used for performing other immobilizations at different enzyme concentration (4×10^{-3} $mg*mL^{-1}$; 8×10^{-3} $mg*mL^{-1}$). The resulting immobilized enzyme was recovered by vacuum filtration and dried overnight at room temperature.

Some immobilizations were also carried out in absence of GLU to evaluate the percentage of the enzyme immobilised by physical adsorption on the zeolite surface.

The amount of the immobilised enzyme was determined by means of the equation of mass balance reported below:

$$m = C_i V_i - (C_f V_f + C_{cs} V_{cs}) \quad (1)$$

where m is the amount of immobilised enzyme, (C_iV_i) is the enzyme present in the initial solution, (C_fV_f) the enzyme present in the final solutions and ($C_{cs}V_{cs}$) that present in the cleaning solution. The enzyme concentration was measured using the Bradford's method [20]. A calibration curve constructed with BSA solutions of known concentration was used in the calculation of protein in the enzyme and cleaning solutions.

2.6 Phosphotriesterase activity for free and immobilized enzyme

For testing the enzyme activity some catalytic reactions were carried out on free and immobilized enzyme.

The catalytic activity of free enzyme was evaluated by monitoring the hydrolysis of the paraoxon-ethyl for producing 4-nitrophenol. In particular, the assay was performed at 25 °C in a mixture of Tris/HCl (0.4 M and pH=8.5) containing 1 mM of paraoxon as substrate. The enzyme concentration was 2×10^{-3}mg*mL^{-1}. The activity was measured by detecting the formation of 4-nitrophenol (reaction product) at 405 nm, in 1-cm path-length cell with a spectrophotometer equipped with a thermo-jacketed cell holder (UV-VIS Spectrophotometer lambda EZ 201, Perkin Elmer). The molar absorption coefficient used for 4-nitrophenol was 19920 M^{-1}cm^{-1} at 25 °C.

The catalytic activity of the immobilized enzyme was measured using a stirred batch reactor containing 30 mg of NaX-GLU-*Sso*Pox W263F particles and paraoxon (1mM) in 30 mL of Tris/HCl (0.4 M and pH= 8.5). Three different enzyme concentration were used (2×10^{-3} mg*mL^{-1}, 4×10^{-3} mg*mL^{-1} and 8×10^{-3} mg*mL^{-1}). Samples were collected in the time from the reaction mixture and analyzed with the spectrophotometer.

The stability of the immobilized PTE was investigated performing seven reactions at an initial enzyme concentration of 2×10^{-3} mg*mL^{-1}and at 25 °C. After each reaction, the PTE-zeolite particles were removed from the batch reactor, rinsed with the buffer solution and stored at 4 °C. After a certain period of time, the immobilized enzyme was immersed in a fresh substrate solution for carrying out a new reaction. For comparison, the stability of free enzyme was also investigated keeping constant the operating conditions.

III. RESULTS AND DISCUSSION

The morphology of the NaX zeolite crystals is shown in Figure 1. The size of the crystals was about 2 µm.

The Si/Al ratio for the NaX zeolite, determined by EDX analysis, was 1.37 (see Figure 2) indicating a hydrophilic nature of this zeolite.

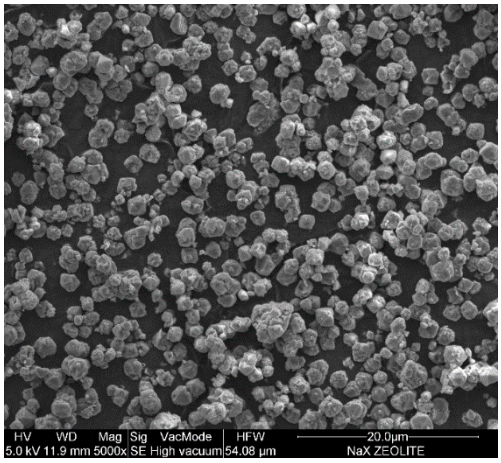

FIGURE 1. SEM image of the NaX zeolite.

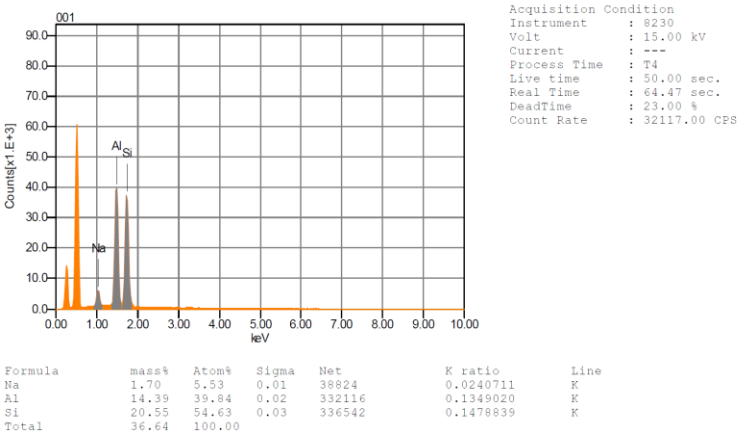

FIGURE 2. EDX analysis of the NaX zeolite.

Glutaraldehyde was used for the NaX zeolite activation [24]. Subsequently, the enzyme was immobilized on the NaX-GLU particles by means of covalent binding, *via* cross-linking. The chemical reaction allowed the formation of a Schiff's base between the enzyme amine and the free group of the glutaraldehyde (see Figure 3).

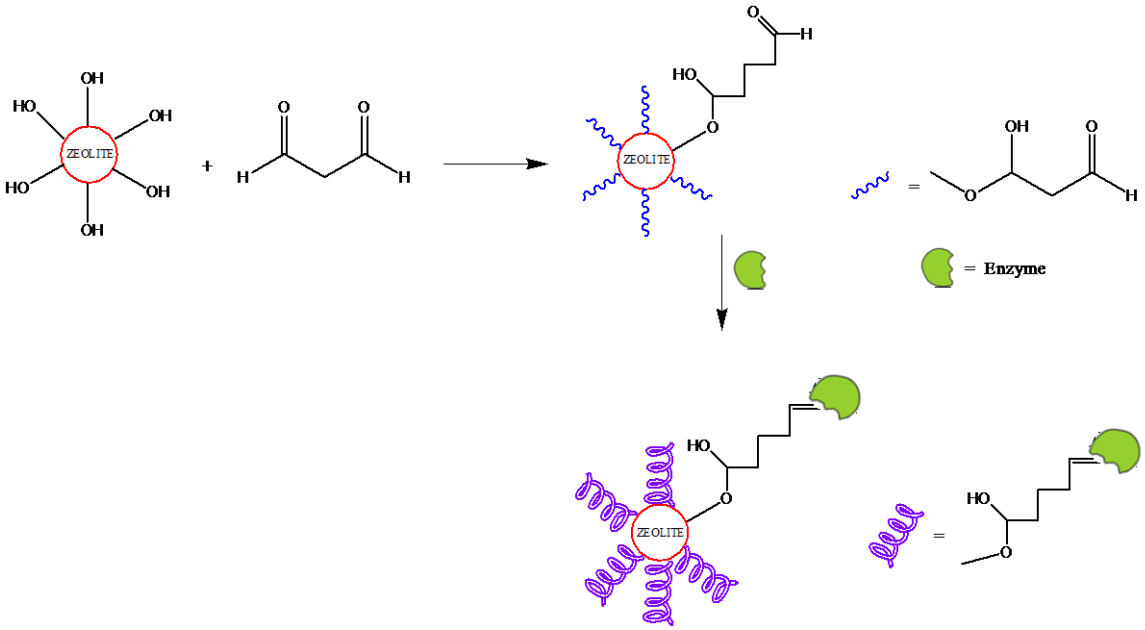

FIGURE 3. Reaction scheme of zeolite activation and enzyme immobilization via cross-linking.

The effect of different GLU concentrations (5 w/v %, 10 w/v %, 15 w/v %, 25 w/v %) on the amount of the immobilized enzyme was evaluated keeping constant the initial enzyme concentration (2×10^{-3} mg*mL^{-1}). The results showed that increasing the GLU content (from 5wt/v % to 10 wt/v%) the amount of immobilized enzyme also increased (see Figure 4). Moving the GLU concentration from 15 w/v % to 25 w/v % the immobilized SsoPox W263F amount reached a plateau. These results can be explained considering that an excessive increase of the GLU concentration leads to a steric hindrance, avoiding a further enzyme immobilization [25]. Other experiments, performed with inactivated zeolite crystals (physical adsorption), indicated a very low quantity of immobilized SsoPox W263F (about 10 %).

The effect of initial enzyme concentrations (2×10^{-3} mg*mL^{-1}, 4×10^{-3} mg*mL^{-1} and 8×10^{-3} mg*mL^{-1}) on the immobilization process was also evaluated by keeping constant the GLU concentration (10 w/v %). As it can be seen in Figure 5, an increase of the initial enzyme concentration permitted to enhance also the immobilized SsoPox W263F amount.

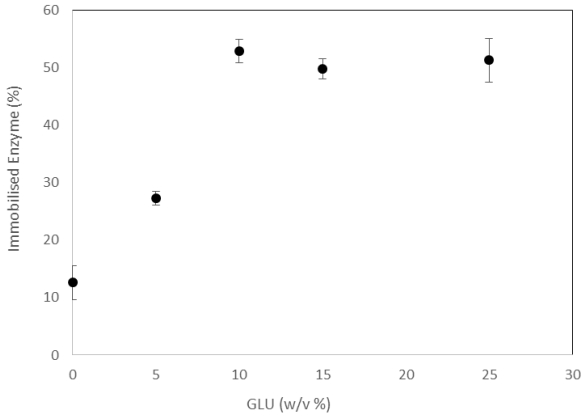

FIGURE 4. Effect of GLU concentration on the amount of immobilized enzyme.

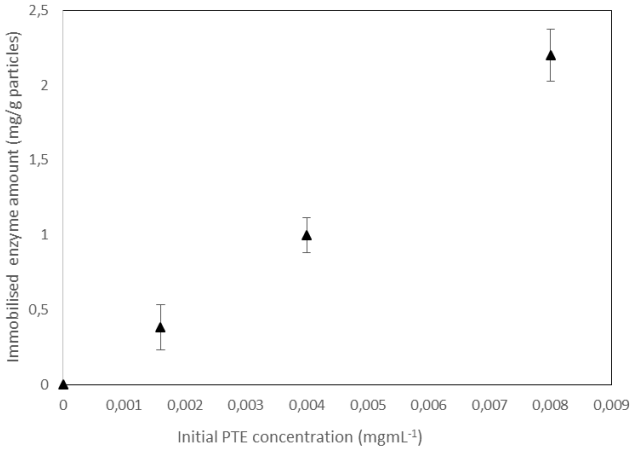

FIGURE 5. Effect of initial enzyme concentration on the immobilized enzyme amount (Operating conditions: pH= 8.5; 60 mg of NaX-GLU particles in 30 mL solution; T=25 °C).

Figure 6 shows that the specific activity of the immobilized *Sso*Pox W263F was independent of the initial enzyme amount and its value was the same of that the free one. This means that the immobilization process did not modify the enzyme properties.

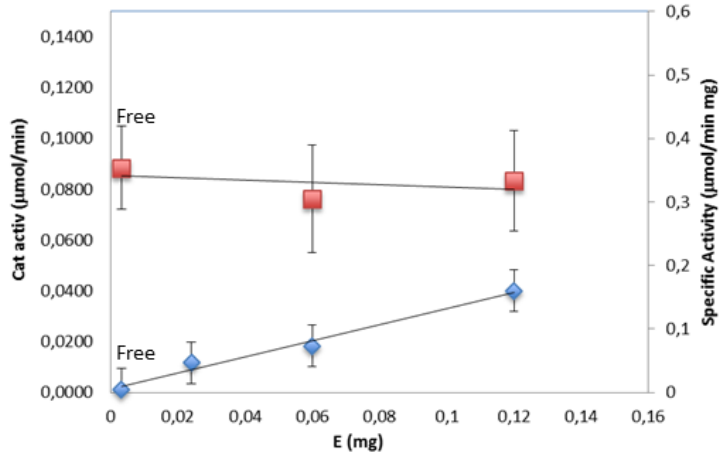

FIGURE 6. Catalytic activity (◆) and specific activity (▭) *vs* the enzyme amount for immobilized and free SsoPox W263F.

The stability of free and immobilized enzyme was also investigated for about two months. The *Sso*Pox W263F in its free (re-suspended in a buffer solution from lyophilized lots) exhibited a specific activity of 0.34 µmol*min^{-1*}mg^{-1} and this value was lost in about two months (see Figure 7). On the other hand, the immobilized form exhibited a slower decay indicating as the immobilization process tends to stabilize the enzyme [26].

An important aspect that must be highlighted is the possibility to recovery and reuse the zeolite crystals for other immobilizations after burning the organic material by thermal treatment at 550°C [9].

This study demonstrated that zeolites can be used as a support for the covalent immobilization of a non-commercial PTE. In particular, it was assessed the possibility to increase the enzyme stability and maintaining the same catalytic activity of its free form. This results is interesting considering that, usually, the immobilization process increases the enzyme stability but causes a reduction of its catalytic activity. Therefore, the NaX-GLU-SsoPox W263F provides an environmental-friendly solution to the problem of pesticides degradation.

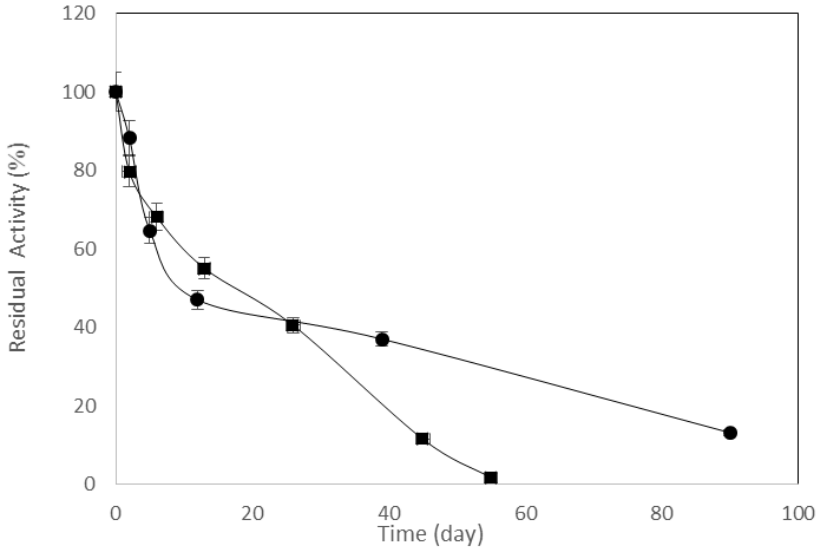

FIGURE 7. Stability of free (■) and immobilized (●) SsoPox W263F *vs* the time.

IV. CONCLUSION

Owing to the toxicity of organophosphate pesticides used in agriculture, is very important to perform the remediation of polluted sites. In particular, the degradation of pesticides with specific enzymes is environmental and socially acceptable. In

this scenario, the present study was focused on the paraoxon degradation by using a PTE-like lactonase covalently immobilized on the NaX crystals. The immobilization process did not change the catalytic properties of the enzyme in terms of specific activity. Besides, the process improved the stability of the enzyme. Considering the peculiar characteristics of the zeolites and the performance of the enzyme immobilized on its surface, this system seems to be a promising functionalized material for application in environmental decontamination.

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REFERENCES

[1] Bouziani M (2007) L'usage immodéré des pesticides. De graves conséquences sanitaires Le Guide de la Médecine et de la Santé, Santémaghreb.

[2] S. Musa, J.W. Gichuki, P.O. Raburu and C.M. Aura,"Risk assessment for organochlorines and organophosphates pesticide residues in water and sediments from lower Nyando/Sondu-Miriuriver within Lake Victoria Basin, Kenya. Lakes & Reservoirs.,"Res. Manage., vol. 16, no. 4, pp. 273-280, September 2011.

[3] I. Horne, T.D. Sutherland, R.L. Harvcourt, R.J. Russel and J.G. Oakeshott, "Identification of an opd (organophosphate degradation) gene in an Agrobacterium isolate," Appl. Environ. Microbiol. Vol. 68, no. 7, pp. 3371-3376. July 2002.

[4] I. Del Giudice, R. Coppolecchia, L. Merone, E. Porzio, T.M.Carusone, L. Mandrich, F. Worek and G. Manco, "An efficient thermostable organophosphate hydrolase and its application in pesticide decontamination,"Biotechnol. Bioeng., vol. 113, pp. 724-734, April 2016.

[5] L. Merone, L. Mandrich, E. Porzio, M. Rossi, S. Muller, G. Reiter, F. Worek and G. Manco, "Improving the promiscuous nerve agent hydrolase activity of a thermostable archaeal lactonase,"Bioresource Technol., vol. 101, pp. 9204-9212, December 2010.

[6] E. Porzio, I. Del Giudice and G. Manco, "Engineering of extremophilic phosphotriesterase-like lactonases for biotechnological applications", Series Title: Grand Challenges in Biology and Biotechnology, Vol. 1, Rampelotto P.H. (Eds): Book Title: Biotechnology of Extremophiles: 978-3-319-13520-5, 331325_1_En, (2016).

[7] Y. Gaoa, Y.B: Truong, P. Cacioli, P. Butler, and I.L. Kyratzis "Bioremediantion of pesticide contaminated water using an organophosphatre degrading enzyme immobilised on nonwoven polyester textile," Enzyme Microb. Technol., Vol. 54, pp. 38-44, January 2014.

[8] C. Algieri, L. Donato and L. Giorno, "Tyrosinase immobilized on a hydrophobic membrane,"Biotechnol. Appl. Biochem., vol. 64, pp. 92-99, October 2015.

[9] C. Algieri, L. Donato, P. Bonacci and L. Giorno, "Tyrosinase immobilised on polyamide tubular membrane for the L-DOPA production: Total recycle and continuous reactor study", Biochem. Engin. J., vol. 66, pp.14-19, July 2012.

[10] L. Donato, C. Algieri, A. Rizzi and L. Giorno, "Kinetic study of tyrosinase immobilized on polymeric Membrane," J. Membr. Sci., vol. 454, pp. 346-350, March 2014.

[11] L. Donato, C. Algieri, V. Miriello, R. Mazzei, G. Clarizia and L. Giorno, "Biocatalytic zeolite membrane for the production of l-DOPA," J.Membr. Sci., vol. 407-408, pp.86-92, July 2012.

[12] K. Chang, R.Z. Huang, S.Y. Lin, S.J. Chiu and J.C. Tsai, "Equilibrium study of immobilized lysozyme on the extrudate-shaped NaY zeolite," Biochem. Engin. J., vol. 28, pp. 1-9, February 2006.

[13] M. Kralj and K. Pavelic, Medicine on a small scale. EMBO Rep., vol. 4, pp. 1008-1012, October 2003.

[14] M. Talebi, S. Vaezifar, F. Jafary, M. Fazilati and S. Motamedi, "Stability Improvement of Immobilized α-amylase using Nano Pore Zeolite," Iran J Biotech., vol. 14 pp. 33-38, March 2016.

[15] Y. Zheng, X. Li and K. Prabir, "Exploitation of unique properties of zeolites in the development of gas sensors," Sensors, vol. 12, pp. 5170-5194, April 2012.

[16] A. Ikhlaq, D.R. Browna and B. Kasprzyk-Hordernc, "Catalytic ozonation for the removal of organic contaminants in water on ZSM-5 zeolites," Appl. Catal. B-Environ.,vol. 154-155, pp. 110-122, October 2014.

[17] A. Kumari, B. Kaur, R. Srivastava and R.S. Sangwan, "Isolation and immobilization of alkaline protease on mesoporous silica and mesoporous ZSM-5 zeolite materials for improved catalytic properties,"Biochem. Bioph. Rep., vol. 2, pp. 108-114, May 2015.

[18] J. Rodriguez, F. Soria, H. Geronazzo and H. Destefanis,"Modification and characterization of natural aluminosilicates, expanded perlite, and its application to immobiliseα-amylase from A. oryzae,"J Mol. Catal. b-Enzym., vol. 133, S259-S270, May 2016.

[19] O.F. Restaino, M.G. Borzacchiello, I. Scognamiglio, E. Porzio, G. Manco, L. Fedele, C. Donatiello, M. De Rosa and C. Schiraldi, "Boosted large-scale production and purification of a thermostable archaeal phosphotriesterase-like lactonase for organophosphate decontamination, "J. Ind. Microbiol. Biotechnol., vol.44, pp. 363-375, March 2017.

[20] M.M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," Anal Biochem., vol. 72, pp. 248-254, May 1976.

[21] T. Montanari, I. Salla and G. Busca, "Adsoprtion of CO on LTA zeolite adsorbents: An IR investigation,"Micropor. andMesopor. Mat.,Vol. 109, pp. 216-222, March 2008.

[22] R.M. Silverstein, G.C. Bassler and T.C Morrill, Spectrometric identification of organic compounds (Fifth Edition). Wiley, New York (1991).

[23] M.S. Bahman, A.S. Mohammad, A. Akbarzadeh, S. Mona, S.H. Gigloo, F. Ali and N.Dariush, "Characteristics of penicillin G acylase immobilized onto iron oxidenanoparticles," Br. Biotechnol. J., vol. 3, pp. 367-376, March 2013.

[24] L. Betancor, F. Lopez-Gallego, A. Hidalgo, N. Alonso-Morales, C. Dellamora-Ortiz, G. Mateo, R. Fernandez-Lafuente and J. Guisan, "Different mechanisms of protein immobilization on glutaraldehyde activated supports: effect of support activation and immobilization conditions," Enzyme. MicrobTechnol, vol. 39, pp. 877-882, August 2006.

[25] T. Bahar, "Clinoptiolite particles as carrier for biocatalysts immobilization: invertase immobilization and characterization," Asian Pac. J. Chem. Eng., vol. 9, pp.31-38, 2014.

[26] A.A. Homaei, R. Sariri, F. Vianello and R. Stevanatojm, "Enzyme immobilization: an update," Chem. Biol., vol.6, pp.185-205, August 2013.

Sustainable Strategy of Charcoal (Panglong Arang) Management in the Bengkalis Regency

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Abstract—

Background and Purpose*: Charcoal is a residue that occurs from the results of decomposition of wood due to heat when most of the chemical components are carbon. One method of making wood charcoal is to use a stove. Charcoal is an important factor in determining the sustainability of the lives of surrounding communities that have long been running. Therefore the purpose of this study is to create a strategy based on the SWOT analysis to see if the charcoal business using mangrove forests can proceed or not.*

Materials and Methods*: Analysis of sustainable development strategies using SWOT Analysis which is one of the analyses used to formulate a strategy in an area of development. This analysis consists of 4 basic elements, namely strengths, weaknesses, opportunities and threats. These four elements come from two main factors: internal factors (strengths and weaknesses) and external factors (opportunities and threats). While the stages in the SWOT analysis.*

Results*: Analysis of public perception of the existence of charcoal trading business in the Bengkalis District gave a "Good response" with a score of 2.66. These results illustrate that the existence of a charcoal trading business does not provide a bad impact as long as it is in a location around a mangrove forest. The results obtained in the SWOT matrix diagram are in the fourth quadrant; show that the priority of the strategy is the Strength (S) and Threats (T) strategies. The components in these two strategies need to get more emphasis and attention so that the existence of a charcoal trading business can be sustainable.*

Conclusion*: The public perception of the charcoal long-standing regulation in the" Bengkalis Regency" is in the "Good category" which is a prerequisite in the development of continuous charcoal. The strategy for sustainable charcoal development is in quadrant IV, namely the S-T strategy.*

Keywords: Panglong Arang, SWOT, Sustainable.

I. INTRODUCTION AND BACKGROUND

Mangroves are found within estuarine and coastal waterways in tropical and subtropical areas. The fauna found in mangroves is therefore also associated with estuarine and coastal waters, making it difficult to separate the importance of mangroves in their life cycle with other features of these water bodies (Manson, 2005). The diversity of the mangrove species growing on this wetland ecosystem is influenced by various factors, including soil conditions, the rate of tides, salinity, levels of inundation (Ari, 2016). Charcoal is a product produced from the carbonization process of materials containing carbon, especially wood biomass. This product is mainly used as an energy source. The process of making charcoal can actually be produced differently, for example ordinary charcoal from combustion is only used as an energy source to produce heat. While charcoal through the activation process its functions can be used for health, pertanian, beauty, electronics, and other things.

Mangroves are very productive ecosystems. Various mangrove products can be produced either directly or indirectly, including: firewood, building materials, household, paper, leather, medicine and fisheries. Seeing the various benefits of mangroves, the levels and rates of rural economies in coastal areas are often very dependent on the surrounding mangrove

habitat (Mariana & Zulkarnaini, 2016). For example, coastal fisheries that are heavily influenced by the presence of mangroves are products that indirectly affect the standard of living and economy of fishing villages.

The history of traditional mangrove utilization by the community for firewood and buildings has been going on for a long time. Even the use of mangroves for commercial purposes such as exports of wood; leather (for dying) and charcoal also have a long history. Mangrove charcoal production has been going on since the past century in Riau and is still ongoing today (Fitri, 2014).

Based on the research of Onrizal & Kusmana (2008) the cause of changes in the mangrove forest cover at the study site was caused by land conversion, logging of mangrove forests for charcoal production carried out by charcoal panglong and lack of public awareness to care for mangrove forests.

II. MATERIALS AND METHODS

Mangrove forests in the community are often referred to as mangroves or brackish forests. However, the mention of mangroves as mangroves seems to be inappropriate because mangroves are one of the names of the species groups in mangroves. Mangrove forests is a group of plant species that grows along the tropical to sub-tropical coastline which has special functions in an environment that contains salt and land forms in the form of beaches with anaerobic soil reactions. In summary, mangrove forests can be defined as a type of forest that grows near tidal areas (especially on protected beaches, lagoons, river estuaries) which are inundated and free of puddles at low tides, whose plant communities tolerate salt (Anwar, 2010).

A Mangrove ecosystem is a system in nature where life takes place which reflects the reciprocal relationship between living things and their environment and between living things themselves, found in coastal areas, affected by tides, and dominated by species of trees or shrubs that are typical and able to grow in salty / brackish waters (Jhonnerie, 2014). Rhizophoraceae species such as R. apiculata, R. Mucronata, and B. gymnorrhiza are good quality firewood because they produce high heat and are durable. The selling price of firewood in the village market is Rp. 13,000 / m^3 which is enough to cook for a month with a family of three children. Mangrove firewood is very efficient, with a diameter of 8 cm and a length of 50 cm is enough for one cooking for 5 people. Firewood is very important for the community, especially from the poor when fuel prices soar (Bidullah, 2013).

Public perception of charcoal (Panglong Arang) is done by using a questionnaire based on a housing quality indicator (HQI) and the Likert Scale (Winarno, 2007).

TABLE 1
PUBLIC PERCEPTION CATEGORIES BASED ON THE HOUSING QUALITY INDICATOR (HQI)

Interval	Categories
1.0 - < 1.75	Really bad (RB)
≥ 1.75 - < 2.5	Bad (B)
≥ 2.5 - < 3.25	Good (G)
≥ 3.25 – 4.0	Really good (RG)

Sustainable development strategy analysis using SWOT Analysis which is one of the analyses used to formulate a strategy in a field of development. This analysis consists of 4 basic elements, namely strengths, weaknesses, opportunities and threats. These four elements come from two main factors: internal factors (strengths and weaknesses) and external factors (opportunities and threats). While the stages in the SWOT analysis are illustrated in Figure 1.

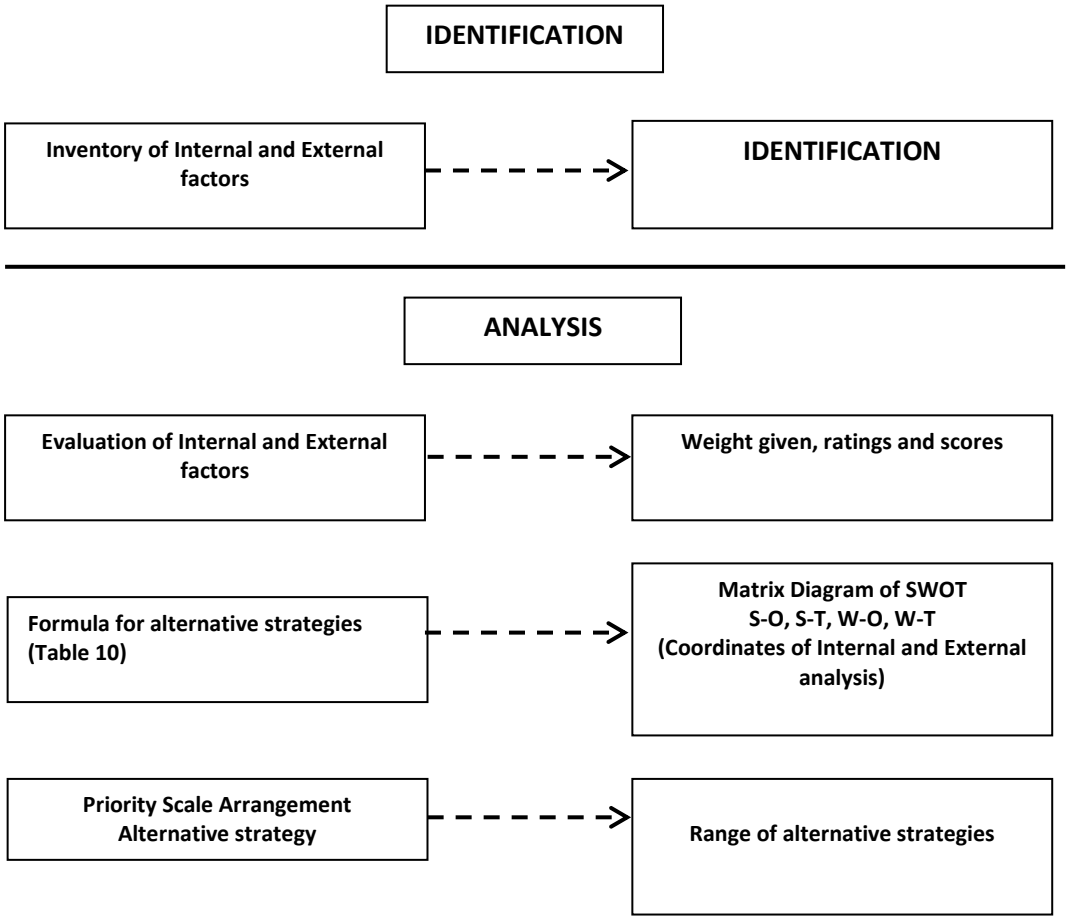

FIGURE 1: Illustration of the stages of the SWOT Analysis in formulating a sustainable development strategy for charcoal in the "Bengkalis Regency" (Rangkuti, 2014)

III. RESULTS

3.1 Results of Community Perception

Analysis of public perception of the existence of charcoal long-standing business in Bengkalis gave a response of Good response with a score of 2.66 as shown in Table 1. These results provide an illustration that the existence of a charcoal trading business does not provide a bad impact during its presence in the surrounding mangrove forest.

TABLE 1
CRITERIA FOR MAKING DECISIONS BASED ON THE PUBLIC PERCEPTION OF PANGLONG ARANG ENTERPRISES IN THE "BENGKALIS REGENCY"

No	Interval	Category	Results	Decision
1	1,0 - < 1,75	Really bad (RB)	2.66	*Good*
2	>= 1,75 - < 2,5	Bad (B)		
3	>=2,5 - < 3,25	Good (G)		
4	>=3,25 - 4,0	Really Good (RG)		

Further study on the existence of the charcoal-burning business according to the perception of the community around the charcoal panglong is in accordance with the results of the question-and-answers that the existence of charcoal panglong economically is quite helpful for the lives of local residents, especially for rural tribes who for years earned a living in the charcoal trading business. Therefore Table 9 reinforces that the existence of charcoal stocks needs to be maintained. On the other hand, the existence of the charcoal trading business is very helpful for the local government in fulfilling employment opportunities for the community around coal trading, the inability of the government to provide new jobs and the limited ability of the community around charcoal to move to other jobs is a big obstacle for the government to close the charcoal trading business, while the daily needs of the community must be fulfilled.

3.2 Results of a Sustainable Charcoal (Panglong Arang) Management Strategy

Due to the existence of the charcoal trading business which been running continuously and the results of public perception show results in the Good category (2.66), then the next step is to make an analysis of the sustainability strategy of the charcoal trading business in the " Bengkalis Regency". The strategy developed in this study is based on the indicator of the management of charcoal panglong business. By making several strategies and alternative strategies in maintaining and developing the charcoal trading business so that the results are expected to be able to provide solutions to the sustainability of the charcoal trading business that are strong in the ecological, economic and social sectors.

This research relies heavily on the initial results obtained from the public's perception of the existence of a charcoal trading business. The purpose of the community perception in the previous analysis (sub-chapter 4.5) is the precondition that needs to be done before the SWOT analysis is carried out, so that the strategy developed really gives a better impact when applied in the field where the charcoal business is located. Community perception can be said to be new findings in the development of SWOT, AHP and the like strategies.

TABLE 2
SCORES IN DETERMINING THE COORDINATES OF THE CARTESIAN INTERNAL STRATEGY (X) AND THE EXTERNAL STRATEGY (Y)

Internal					**X**
Strategy		**Weight**	**Rating**	**Score**	
Strength (S)	S1	0.199	3.7	0.736	
	S2	0.152	3.3	0.502	
	S3	0.164	3.6	0.589	
	S4	0.164	3.5	0.573	
	S5	0.164	3.5	0.573	
	S6	0.158	3.2	0.505	
				3.478	
					0.02
Weaknesses (W)	W1	0.169	3.3	0.558	
	W2	0.174	3.9	0.677	
	W3	0.155	3.9	0.604	
	W4	0.174	3.4	0.591	
	W5	0.164	3	0.493	
	W6	0.164	3.1	0.509	
				3.432	
External					**Y**
Strategy					
Opportunities (O)	O1	0.202	3.4	0.687	
	O2	0.176	2.6	0.458	
	O3	0.176	2.8	0.493	
	O4	0.083	2.8	0.232	
	O5	0.187	2.7	0.504	
	O6	0.176	3.4	0.599	
				2.973	
					-0.26
Threats (T)	T1	0.170	3.7	0.628	
	T2	0.160	3.3	0.529	
	T3	0.151	3.7	0.558	
	T4	0.189	3.5	0.660	
	T5	0.179	3.5	0.627	
	T6	0.151	3.2	0.483	
				3.487	

A strategy cannot be developed if the results of the initial study do not support the next step. In this study a strategy can be developed because it has gained initial perceptions from the community around the charcoal business in the Good category. Based on the results in Table 10, they show the x and y coordinates, so it is very easy to put positions in each quadrant on the coordinate system of the Cartesian SWOT strategy.

IV. DISCUSSION

4.1 Community Perception

The charcoal "Panglong Arang" that has been built so far provides hope for the welfare of the community when they need to make a livelihood in order to fulfill their living needs, while the government's effort to provide a decent life in the aspect of employment is not yet sufficient. The proof of the response of the community is a score of 2.66 (Good), which illustrates that they still hope that the existence of a charcoal trading business has been proven to be able to provide a solution for the survival of their families. Priceless gratitude from the managers (owners) of the charcoal companies who provide sources of livelihood like those that are interrelated. The results of community perceptions in this study illustrate that the existence of the charcoal trading business only needs to be better managed by not damaging the surrounding environment so as to give great hope in the development in the ecological, economic and social sectors.

It can be said that in this case the mangrove forest is the main source of the surrounding community in running the economy and the survival of the community through mediation of the charcoal trading business, just how can this charcoal business be developed to become a sustainable charcoal business. Another effort that can be developed in this study is based on the results obtained from the questionnaire with a score of 2.66 (Good), giving the researcher an idea to develop a sustainable development strategy for charcoal in the "Bengkalis Regency".

4.2 Sustainable Charcoal (Panglong Arang) Management Strategy

The results obtained in the SWOT matrix diagram in Figure 2 are in the fourth quadrant, this shows that the priority strategy is the Strength (S) and Threats (T) strategies. The components in these two strategies need to get more emphasis and attention so that the existence of a charcoal trading business can run well and be sustainable. Based on this value, where the threat factor is slightly greater than the strength factor (0.02; -0.26), this illustrates that in addition to the charcoal trading business permit, environmental damage and the existence of land conversion function constitute the biggest threat to the sustainability of the charcoal trading business. For example, the existence of land conversion into a shrimp pond has penetrated the location of a charcoal trading business.

The existence of shrimp farming business uses a small amount of land, so the supply of raw materials for charcoal, especially white mangroves (Rhizophora apiculata) is very difficult to find. To overcome this problem it is necessary to utilize the existing strengths as stated in the Strength (S) component. To reduce human activity in exploiting mangrove forest, it is necessary to do mangrove forest management which has legal force. Given the importance of the role of mangrove for coastal communities, either directly or indirectly, these natural resources must be managed as well as possible. The essence of good mangrove forest management is to provide opportunities for people living around the coast to participate and cooperate with government officials (Mita, 2017).

According to Costanza et al. (1997) calculated that the economic value of estuaries in terms of services and natural capital per hectare was the highest of all ecosystems. Tropical mangrove systems, in particular, are zones of high productivity (Blaber, 2000), and assuming that most of the fisheries productivity is closely linked to mangroves, a number of recent studies have emphasized the economic value of mangroves, especially in the developing world (Hamilton et al., 1989; Barbier and Strand, 1998; Nickerson, 1999; Barbier, 2000).

The strength component consists of permits to manage environmentally friendly mangrove forests by the Regulations of the Regent and the Ministry of the Environment, community support based on the results of perceptions given, the equipment used adheres to Local Knowledge and Ethno technology which are very environmentally friendly.

TABLE 3
RANKING AND PRIORITY STRATEGY DETERMINATION

Quadrant	Position point		Area matrix	Ranking	Strategic Priority
I	3.48	2.97	10.34	3	S-O
II	3.43	2.97	10.20	4	W-O
III	3.43	3.49	11.97	2	W-T
IV	3.48	3.49	12.13	1	S-T

Based on Table 3, it can be seen that the highest strategic priority is in quadrant IV, namely S-T. In this strategy analysis, it can be detailed in the S-T components that need to be a concern in the continuous development of the charcoal-burning business in the "Bengkalis Regency". The strength factors consist of:

1. Fertile areas with high biodiversity
2. The condition of river waters that have not been polluted by industrial waste
3. The availability of cooperative groups in the management of charcoal and mangrove forests
4. Market demand for both local and international charcoal is quite high
5. Aquatic resources for the community of freshwater organisms
6. There are local government regulations and ministerial regulations in coastal mangrove management.

While the threat factor (Threats) consists of:

1. Commercial charcoal businesses create waste that threatens the sustainability of mangroves
2. Panglong charcoal business threatens the existence of flora and fauna
3. Efforts to develop long-term businesses that benefit third parties (foreigners) such as charcoal smuggling
4. The occurrence of land conversion such as large shrimp ponds can threaten the destruction of mangrove forests and charcoal trading businesses
5. The high intensity of law violations in the use of forests and rivers
6. Excessive exploitation of mangrove resources.

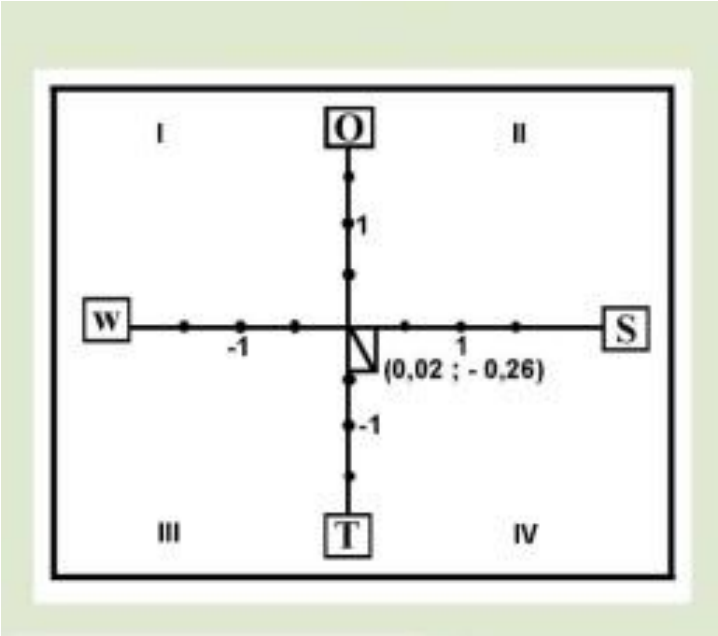

FIGURE 2. SWOT matrix diagram for the management of charcoal long business in Bengkalis Regency

These twelve factors can be seen in Figure 2 and 3. Figure 2 is a SWOT matrix diagram with priority and ranking scales in quadrant IV where the existence of a long-term business really needs an emphasis on quadrant IV because the existence of a charcoal trading business needs more attention because the results of the questions and answers with the surrounding community give the same picture of their desire to maintain this charcoal business. If these twelve components can be localized, the existence of a charcoal trading business in an effort to sustain the district economy and environmental sustainability, it will run well. With the spatial planning will provide the regularity in the implementation of development and will avoid the occurrence of abuse in the allocation of space and excessively use of resources without regard to aspect of sustainability (Marliana, 2012).

In the general strategy matrix diagram, the overall factors that need to be improved in the management of sustainable charcoal business are illustrated. Furthermore, it can be made a determinant of the success of the sustainable development strategy of charcoal long-standing business in Bengkalis Regency. Determinants in the sustainable development of charcoal are:

1. The existence of local government regulations and ministerial regulations in coastal mangrove management.
2. The high intensity of law violations in the use of forests and rivers

While the sustainable charcoal business development strategy model can be seen in Figure 16. In this figure there is a new model in the use of SWOT strategies that must meet the prerequisites before the SWOT strategy is used in the sustainable development of charcoal trading business. A circle consisting of 4 colors is a decisive circle that is associated with the value of public perception as a first step in developing a strategy.

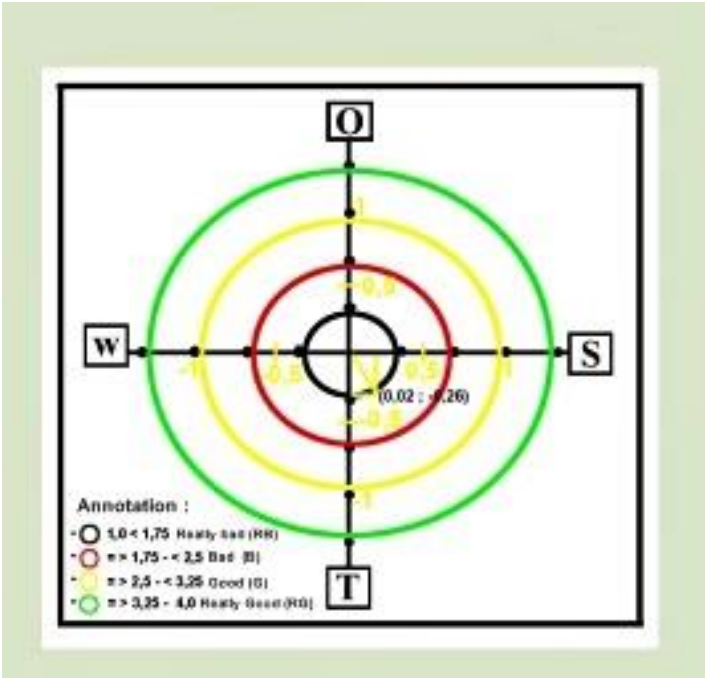

FIGURE 3. The matrix diagram of the development strategy for charcoal long business in the "Bengkalis Regency"

This form of sustainable development of charcoal trading is a novelty in the development of strategies, because if the results of the calculation of public perception values are in the black-red circle then the strategy cannot be developed because it does not get support from the local community where they are. Whereas if the perception value of the community is in the yellow-green circle then the strategy can be continued, meaning that the initial step in the development of charcoal trading business gets support from the community in accordance with the existing perception value.

V. CONCLUSION

The public perception of the charcoal long-standing regulation in the "Bengkalis Regency" is in the Good category which is a prerequisite for the development of continuous coal charcoal. The strategy for sustainable charcoal development is in quadrant IV, namely the S-T strategy.

SIGNIFICANCE STATEMENT

This study found a new method in developing management strategies using the SWOT analysis, namely the existence of prerequisites that must be met before a strategy is developed, namely community perception. If the decision criteria are in the category of Good (G) or Really Good (RG) then a strategy can be developed or continued. In this study, people's perceptions are in the Good category.

REFERENCES

[1] Anwar, C & Gunawan, H. (2010). *Peranan Ekologis dan Sosial Ekonomis Hutan Mangrove Dalam Mendukung Pembangunan Wilayah Pesisir*. Makalah Utama pada Ekspose Hasil-hasil Penelitian : Konservasi dan Rehabilitasi Sumberdaya Hutan, Padang.

[2] Ari,S, Fajar, R, Rudianda, S, Sigit, S, & Koichi Yamamoto. (2016). Investigation of Mangrove Biomass Potential in Indragiri Hilir Wetlands, Indonesia. *International Journal of Environmental Science and Development*, Vol 7. No 12, Hal : 902-907.

[3] Barbier, E. B. 2000. Valuing the environment as input: review of applications to mangrove fishery linkages. Ecol. Econ. 35: 47–61.

[4] Barbier, E. B I. & Strand. 1998. Valuing mangrove-fishery linkages. Environ. Resour. Econ. 12: 151–166.

[5] Bidullah, Y.I., Isa, I., & Alio La. 2013. *Uji Daya Serap Arang Aktif dari Kayu Mangrove Terhadap Logam Pb dan Cu*. Thesis FMIPA Universitas Negeri Gorontalo. Tidak diterbitkan.

[6] Blaber, S. J. M. 1980. Fishes of the Trinity Inlet system of North Queensland with notes on the ecology of fish faunas of tropical Indo-Pacific estuaries. Aus. J. Mar. Freshw. Res. 31: 137–146.

[7] Costanza, R., R. d'Arge, R. de Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. O'Neill, J. Paruelo, R. G. Raskin, P. Sutton, and M. van den Belt. 1997. The value of the world's ecosystem services and natural capital. Nature 387: 253–260.

[8] Fitri, R.Y (2014). Kebijakan Pemerintah Terhadap Pelestarian Hutan Mangrove Di KecamatanTebingTinggiKabupaten Bengkalis. *Jom FISIP*Vol. 1 No. 2. Hal: 1-15.

[9] Hamilton, L., J. Dixon, and G. Miller. 1989. Mangroves: an undervalued resource of the land and sea. Ocean Yearb. 8 : 254–288.

[10] Jhonnerie, R., Siregar, V.P., Nababan, B., Prasetyo, L.B., & Wouthuyzen, S. (2014). Deteksi Perubahan Tutupan Mangrove Menggunakan Citra Landsat Berdasarkan Klasifikasi Hibrida di Sungai Kembung, Pulau Bengkalis, Provinsi Riau. *Jurnal Ilmu dan Teknologi Kelautan Tropis.* Vol 6 No 2 : 491 – 506.

[11] Manson, F.J., N.R Loneragan, G.A silleter, and S.R. Phinn. (2005). "An Evaluation of the Evidence for Lingkages Between Mangroves and Fisherris: A Synthesis of the Literature and Identification of Research Directions". Dalam *Oceanography and Marine Biology: An Annual Review* 43: 485-515.

[12] Marliana, D., Sarwono., & Rozikin, M (2012). Kebijakan Pengelolaan Wilayah Pesisir Berbasis Sustainable Development Di Kabupaten Sampang (Studi Pada Bappeda Kabupaten Sampang). *Jurnal Administrasi Publik* (JAP), Vol.1. No.3 : 80-86.

[13] Mita. R. (2017). Akit Tribe and Existence of Mangrove Forest in Berancah Village, Bengkalis, Indonesia Sociology Department, Universitas Riau, Pekanbaru, Indonesia.

[14] Nickerson, D. J. 1999. Trade-offs of mangrove area development in the Philippines. Ecol. Econ. 28: 279–298.

[15] Onrizal & Kusmana, C. (2008). Studi Ekologi Hutan Mangrove di Pantai Timur Sumatera Utara. *Biodiversitas*. Vol 9, No 1 : 25-29

[16] Rangkuti,F. (2014). Analisis SWOT: teknik membedah kasus bisnis (reorientasi konsep perencanaan strategis untuk menghadapi abad 21). Buku. PT.Gramedia Pustaka Utama. Jakarta. 246 p.

[17] Renny Eka Putri.R.E, & Andasuryani (2017). Studi Mutu Briket Arang Dengan Bahan Baku Limbah Biomassa. Jurnal Teknologi Pertanian Andalas Vol 21. No 2. Issn 1410-1920, Eissn 2579-4019.

[18] Salim. R (2016). The Quality and Characteristics of Teak (Tectona grandis) Charcoal Made by Mixed Carbonisation in Drum Kiln. Jurnal Riset Industri Hasil Hutan Vol 8. No 2, Hal : 53 – 64.

[19] Tambunan, R., R., Harahap, R.H., & Lubis, Z. (2005). Pengelolaan Hutan Mangrove di Kabupaten Asahan. *Jurnal Studi Pembangunan* Vol 1. No 1 : 55- 69.

[20] Winarno, B. (2007) *Kebijakan Publik: Teori dan Proses (edisi revisi)* Media Pressindo; Jakarta.

[21] Zulkarnaini & Mariana (2016). Economic Valuation of Mangrove Forest Ecosystem in Indragiri Estuary. *International Journal Oceans and Oceanography*. Vol 10. No 1 : 13-17.

In-Vitro Management of *Erwinia carotovora* the Causal Organism of Potato Soft Rot Disease

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Abstract*—In vitro efficacy six chemical substance were evaluated against Erwinia carotovora the causal organism of potato soft rot disease. E. carotovora was isolated from diseased potato tubers by dilution plate technique and different biochemical and pathogenicity test were performed to confirm the bacterial species. Six chemicals viz. Copper Oxychloride @ 0.2%, Mancozeb @ 0.2%, Boric acid @ 0.1%, Kasugamycin @ 0.02%, Carbendazim @ 0.3% and Sodium Hypochlorite @ 0.2% were tested against E. carotovora subsp. carotovora by well diffusion method. For each treatment there were four replications and tested chemical volume was 100 µl. Data were recorded up to five days of incubation. Maximum zone of inhibition (mm) was obtained after 48 hours of incubation with Copper Oxychloride (30.35 mm), followed by Mancozeb (20.15 mm), Boric acid (19.15 mm) and Kasugamycin (16.28mm). Copper Oxychloride produced the maximum growth inhibition (33.72%) of the pathogen, on the other hand Sodium Hypochlorite (2.68%) did not efficiently inhibit the growth of E. carotovora. Copper Oxychloride proved to be the best chemical followed by Mancozeb under in-vitro management against E. carotovora subsp. carotovora.*

***Keywords*—Soft rot, in-vitro, chemicals, zone of inhibition.**

I. INTRODUCTION

Potato (*Solanum tuberosum* L.) is a starchy tuber crop belongs to the family Solanaceae. Potato possess number four position food crop after wheat, maize and rice in the world (Douches *et al.*, 1996) [1]. In Bangladesh the average yield of potato has been estimated 82, 05, 470 metric tons in the year 2011-2012 (BBS, 2012) [2]. Still potato production is quite low in comparison to that of the leading potato growing countries of the world.

Among many pathogenic bacteria *E. carotovora* subsp. carotovora causing potato soft rot disease is considered as most important disease (Akbar *et al.*, 2014) [3]. It is an important post-harvest disease which cause huge losses in stored potatoes if not properly managed. It has been estimated that every year 22% of potatoes are lost due to fungal, viral and bacterial diseases and pests, which is comparable to a yearly loss of more than 65 million tones and bacterial soft rot contributes it as much as 50% alone of the total potato production (Czajkowski *et al.*, 2011) [4]. The effect of soft rot disease is more prominent in the countries where suitable storage facilities are insufficient.

Generally chemical substances are not prescribed for the management of bacterial disease because it has high chance of lingering issue on human wellbeing and negative impression on the environment. However, many researchers evaluated various chemicals in order to control the soft rot bacteria. Copper-based compounds were found more effective against *E.carotovora* among numerous chemicals *in-vitro* (Rashid *et al.*, 2013) [5]. Acetic acid, boric acid and bleaching powder considerably reduced the infection rate, loss in weight and percentage of disease reduction against *E. carotovora* subsp. *carotovora* (Rahman *et al.*, 2017) [6]. The antibiotics had a substantial effect on plant pathogenic microorganisms which formed cell wall degrading enzymes (Alice and Sivaprakasam, 1995) [7].

Considering the great economic losses, the present investigation was undertaken to isolate and identify of causal organism and to evaluate the efficacy of six different chemicals against *E. carotovora* subsp. *carotovora* under *in-vitro* condition.

II. MATERIAL AND METHOD

The experiment was done in the Molecular Plant Pathology Laboratory of Department of Plant Pathology, Sher-e-Bangla Agricultural University. The experiment comprised of the purification and identification of the bacteria of potato soft rot disease and evaluation of efficacy of six chemicals against the causal organism under *in-vitro* condition.

2.1 Purification of the bacteria from diseased tubers

The potato tubers were collected from various places in Dhaka district. The pathogen was isolated by dilution plate method. Diseased tubers were surface sterilized with 95% ethanol for 3 mins, rinsed entirely with sterile water. The diseased parts of potato tuber were separated and macerated in sterile water to make a bacterial suspension. Tenfold serial dilution was made from the stock solution. 0.1 ml of each dilution was put on a nutrient agar (NA) plate and distributed using glass rod. Similar procedure was done three times (Goszczynska and Serfontein, 1998) [8] and were incubated at 30±1 ^{o}C for two days. A part of a well isolated typical colony was taken using a sterile wire loop and streaked on fresh NA plate to get pure culture.

2.2 The bacteria identification

The bacteria were identified by the following tests:

2.2.1 Grams staining reaction

A well isolated young colony was smeared on a glass slide followed by heat fixation. After a series of Grams staining reaction, described by Gerhardt (1981) [9] at 100x magnification the slide was viewed.

2.2.2 Biochemical Tests

In KOH solubility test, the bacterial colony was mixed with 3% KOH solution and any alteration in the consistency was recorded (Suslow *et al*., 1982) [10]. During catalase test 2-3 drops of afresh ready 3% H_2O_2 (Hydrogen peroxide) was placed on two days old pure bacterium culture on nutrient agar plate and observed whether the bacteria formed bubbles within a few seconds or not (Schaad, 1988) [11]. In oxidase test the bacteria colony was smeared on filter paper containing NNN'Ntetramethyl-p-phenylene-diamine-dihydrochloride and color changes was recorded (Kovacs, 1956) [12]. During gelatin liquefaction test nutrient broth containing 12% gelatin with bacterial *E. carotovora* culture incubated at 30 ^{o}C for 1-2 days followed by 5 ^{o}C in refrigerator for 15 minutes and it was observed whether the bacteria liquefied gelatin or not (Salle, 1961) [13]. In starch hydrolysis test, bacterium pure culture was streaked on the central of NA plate containing 2% soluble starch followed by incubation then the plate was awash with lugol's iodine solution. The existence or non- existence of clear zones in stained media was noted. (Cowan, 1974) [14].

2.2.3 Pathogenicity test:

Bacterial culture was suspended into sterile distilled water to make bacterial cell suspension. 0.5 ml suspension was placed into a hollow cut in the healthy potato tuber. Growth of rot on the tuber was viewed for 1-2 days after incubation at 28±2 °C (Prashant B Sandipan, 2014) [15]. Bacteria were re- isolated from macerated tissue and compared with the original isolate of inoculated pathogen (Shashirekha *et al*., 1987) [16].

2.3 *In-vitro* management of *Erwinia carotovora* subsp. *carotovora*

Six selected chemicals viz. Copper oxychloride, Mancozeb, Boric acid, Kasugamycin, Carbendazim and Sodium hypochlorite were tested against the test bacterium *E. carotovora* subsp. *carotovora* by well diffusion method measuring the zone of inhibition. Two wells were made with a cork borer of 5 mm in diameter in the individual NA plate and the pure culture of *E. carotovora* subsp. *carotovora* was streaked thoroughly on it with a sterile loop. One well was filled with definite concentration chemical suspension with 100 µl volume and other well was filled with sterile water. Each combination of pathogen, chemical and sterile water was replicated four times and plates were incubated at 30±1ºC. Inhibition zone around the wells was measured by observing the growth of bacterial and noted each day for 5 days.

Growth inhibition percentage (%) was determined using the formula modified by Amadioha (2004) [17] as

$$\text{inhibition} = \frac{dc - dt}{dc} x100\% \quad (1)$$

Here,

dc = Diameter of colony in control

dt = Diameter of colony in treatment

2.4 Data analysis

Collected data during experiment period were tabulated and analyzed using computer software MSTAT-C.

III. RESULTS AND DISCUSSION

3.1 Identification of bacteria from colony morphology

The colonies of bacteria *Erwinia carotovora* subsp. *carotovora* were found creamy white, round, slightly raised, smooth with entire edges, small to moderate large on NA media (Figure 1). Corresponding types of colonies were found by Opara and Agugo, 2014 [18].

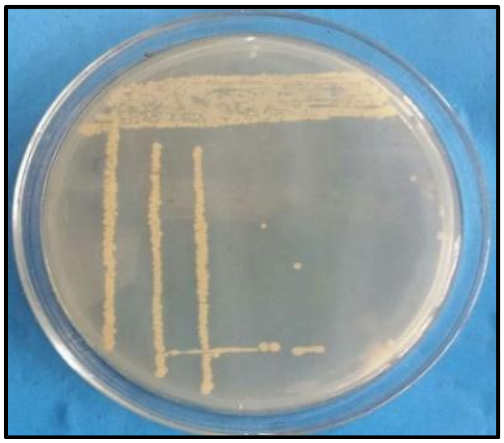


FIGURE 1. Pure culture of bacteria *Erwinia carotovora* subsp. *carotovora*

3.2 Identification of bacteria from biochemical characters

The isolated bacteria, *Erwinia carotovora* subsp. *carotovora* was confirmed by different biochemical tests (table 1). The bacteria were gram negative as they resulting red color after a series of Gram reaction test. In Gram differentiation test or KOH solubility test, the bacteria formed a mucoid strand when lifted with the help of toothpick. The bacteria formed bubbles resulting positive catalase test. The bacteria formed dark purple color in oxidase test. In gelatin liquefaction test the bacteria liquefied gelatin. A clear zone appeared around the colony, in starch hydrolysis test.

3.3 Pathogenicity test

Artificially inoculated potato tubers yielded the bacterial colonies alike to the genuine ones resulting positive pathogenicity test. Based on the morphological, biochemical and pathogenicity test, the pathogen was identified as *E. carotovora* subsp. *carotovora*.

TABLE 1
CHARACTERISTICS OF ISOLATED BACTERIA *E. CAROTOVORA* SUBSP. *CAROTOVORA* TO DIFFERENT TESTS ARE LISTED BELOW

Name of tests	Reaction
Gram staining	-
Gram differentiation test (KOH solubility test)	+
Gelatinliquification test	+
Starch hydrolysis test	+
Catalase test	+
Oxidase test	+
Pathogenicity test	+

3.4 Management of *E. carotovora* subsp. *carotovora* under *in-vitro* condition

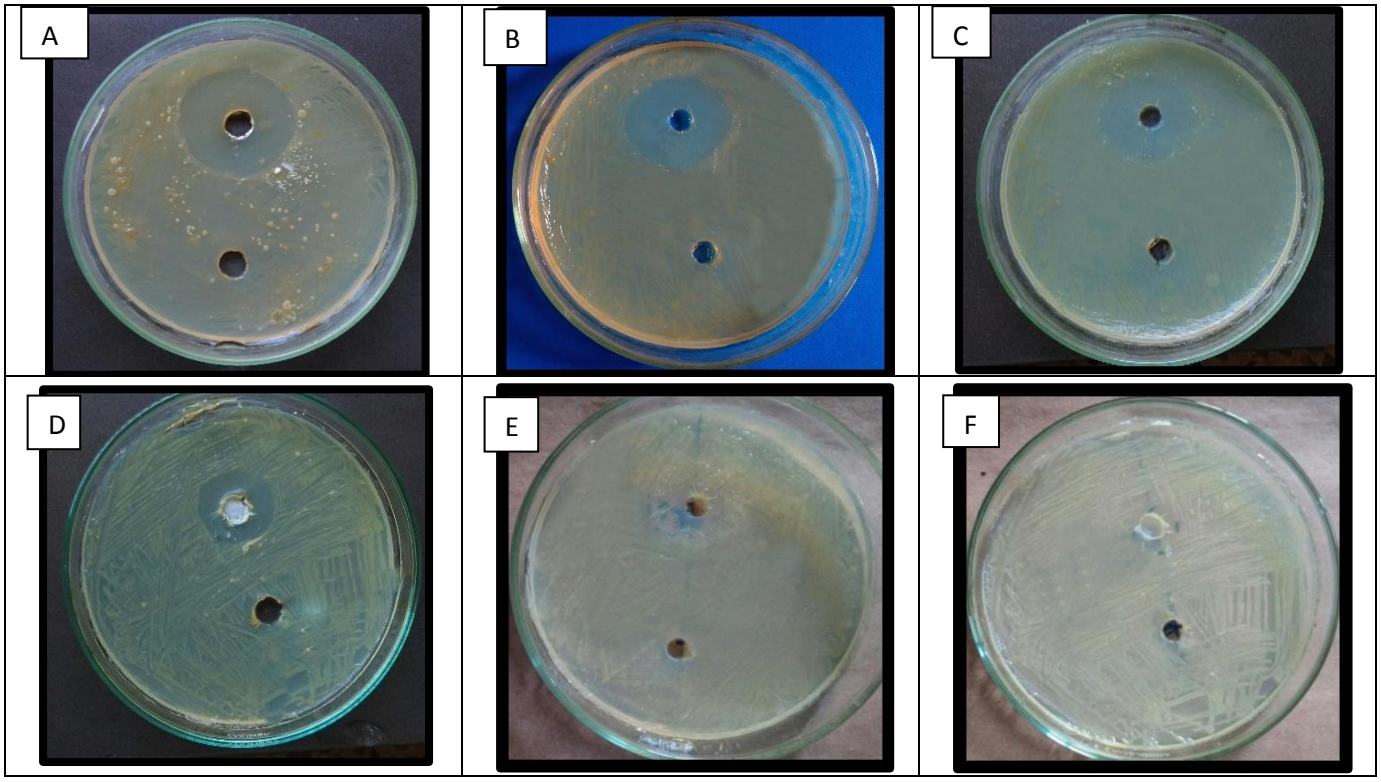
In-vitro evaluation of six different chemical substances were studied against *Erwinia carotovora* subsp. *carotovora* and found significant variations in terms of inhibition zone of isolated bacteria (Table 2 and Figure 2). Among the six chemicals, Copper oxychloride at 0.2% showed the maximum inhibition zone (30.35 mm) after two days of incubation followed by Mancozeb (20.15mm). Boric acid at 0.1% and Kasugamycin at 0.02% showed moderate inhibition zone 19.15mm and 15.08mm, respectively. Sodium hypochlorite at 0.2% showed the minimum inhibition zone 2.42mm.

TABLE 2

ACTIVITY OF SIX CHEMICALS AGAINST *ERWINIA CAROTOVORA* SUBSP. *CAROTOVORA* THE RESPONSIBLE ORGANISM OF POTATO SOFT ROT DISEASE *IN VITRO*

Chemicals	Con. (%)	Volume µl	Inhibition Zone (mm)				
			24h	48h	72h	96h	120h
Copper oxychloride	0.2	100	27	30.35	28.25	26.05	22.28
Mancozeb	0.2	100	18.4	20.15	18.48	16.18	13.65
Boric Acid	0.1	100	16.83	19.15	17.63	15.83	12.83
Kasugamycin	0.02	100	14.00	15.08	13.88	11.98	9.68
Carbendazim	0.3	100	2.39	3.83	1.28	0	0
Sodium hypochlorite	0.2	100	1.15	2.42	0.675	0	0

Note: Each data represents the mean of four replications



Clear zone indicates the inhibition zone

FIGURE 2: Screening of six chemical substance against *Erwinia carotovora* subsp. *carotovora* (A) Copper oxychloride (B) Mancozeb (C) Boric Acid (D) Kasugamycin (E) Carbendazim and (F) Sodium hypochlorite after 48 hours of incubation.

Efficiency of chemical substance used for this experiment was studied. Different chemicals showed different effects on growth inhibition of *E. carotovora* subsp. *carotovora*.

TABLE 3

EFFICACY OF SIX CHEMICALS IN INHIBITION OF GROWTH OF *E. CAROTOVORA* SUBSP. *CAROTOVORA*

SI No.	Chemical substance	Growth inhibition (%) of *E. carotovora* at 48 hours after incubation
1.	Copper oxychloride	33.72
2	Mancozeb	22.38
3	Boric Acid	21.28
4	Kasugamycin	16.76
5	Carbendazim	4.26
6	Sodium hypochlorite	2.68
7	Control	0.00

The results presented in Table 3 revealed that Copper oxychloride produced the maximum growth inhibition (33.72%) of the pathogen after two days of incubation and was statistically superior over rest of the chemicals tested. Other chemicals viz., Sodium hypochlorite (2.68%) did not effectively inhibit the growth of *E. carotovora* subsp. *carotovora.*

IV. CONCLUSION

Bacteria can multiply very fast and produce disease in favorable condition and the most serious aspect is that there are hardly any prospects to manage bacterial pathogens on potato. Employ of chemical substance free from health risk can be conducive and suitable way to manage potato soft rot disease. Among the chemicals tested in this experiment Copper oxychloride @ 0.2% was found most effective against the bacteria, other chemicals also had moderate effect against this bacterium *in vitro*. A through and large-scale research is required to find out an effective method to control soft rot of potato.

REFERENCES

[1] Douches DS, Maas D, Jastrzebski K, Chase RW. Assessment of potato breeding progress in the USA over the last century. *Crop Science*. 1996; 36: 1544–52.

[2] BBS 2012: Statistical Year Book of Bangladesh. Statistics Division, Agriculture Wing, Ministry of Planting, Govt. of the People's Republic of Bangladesh, Dhaka. P.60

[3] Akbar A, Din S, Ahmad M, Khan G, Ala, S. Effect of Phytobiocides in Controlling Soft Rot of Tomato. *J. Nat. Sci. Res*. 2014; 4:11.

[4] Czajkowski R, Perombelon MCM, vanVeen JA, van der Wolf JM. Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: a review. *PlantPathology*. 2011; 10: 1365- 3059.

[5] Rashid M, Chowdhury MSM, Sultana N. *In-vitro* Screening of some Chemicals and Biocontrol Agents against *Erwinia carotovora* subsp. *carotovora*, the Causal Agent of Soft Rot of Potato (*Solanum tuberosum*). *The Agriculturists*. 2013; 11(2): 1-9.

[6] Rahman MM, Khan AA, Mian IH, Akanda AM, Alam MZ. Effect of some chemicals on incidence of potato soft rot disease in Bangladesh. *Bangladesh J. Sci. Ind. Res*.2017; 52(2): 135-140.

[7] Alice D. and Sivaprakasam K. Antibiotics and garlic clove extract inhibitory agents of cell wall degrading enzymes. *Hindustan Antibiotics Bulletin*. 1995; 37: 44-47.

[8] Goszczynska T, Serfontein JJ. Milk tween agar, a semiselective medium for isolation and differentiation of *Pseudomonas syringae*pv. *syringae*, *Pseudomonas syringae*pv. *phaseolicola* and *Xanthomonas axonopodis*pv. *phaseoli*. *J. Microbiol. Method*.1998 32(1): 6572.

[9] Gerhardt P. Manual of methods of general bacteriology. American Society of Microbiology Washington, D.C. 1981, p. 127

[10] Suslow TV, Schroth MN, Isaka M. Application of a Rapid Method for Gram Differentiation of Plant Pathogenic and Saprophytic Bacteria without Staining. The American Phytopathological Society. 1982; 10(1094): 72-917.

[11] Schaad NW. Laboratory guide for identification of plant pathogenic bacteria. 2nd ed. American Phytopathological Society, St. Paul, Minnesota. 1988; 185-197.

[12] Kovacs N. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature. 1956; 178: 703.

[13] Salle AJ. Fermentation of carbohydrates and related compounds. In: Laboratory manual on Fundamental Principles of Bacteriology. 5th ed. McGraw Hill Book Company, Inc. New York, USA. 1961; 94-98.

[14] Cowan ST. Cowan and Steel's Manual for the Identification of Medical Bacteria, 2nd ed. Cambridge University Press, Cambridge. 1974; 67-83.

[15] Prashant B Sandipan. Isolation, Morphology, Identification of Pathogens and Confirmation of Pathogenicity of Dry Rot, Charcoal Rot and Soft Rot Pathogens of Potato. *IJLBPR*. 2014; 3(4).

[16] Shashirekha MN, Karanth NGK and Narasimham P. Surface microflora of seed potatoes (*Solanum tuberosum* L., Kufri Jyoti): Isolation and identification of organisms responsible for spoilage of potatoes grown at Devanahalli. *Journal of Food Science and Technology*. 1987; 24:261-263.

[17] Amadioha AC. Control of Black rot of potato caused by *Rhyzoctonia bataticola* using some plant leaf extracts. Arch. Phytopathol. Plant Pret. 2004; 37:111-117.

[18] Opara EU and Agugo BA. Control of Posthavest Rot of Sweeet potato (*Ipomoea batatas* Lam.) Tuber *in Vitro* and *in Vivo*. *Journal of Agriculture and Sustainability*.2014; 6 (1): 50-68.

Constraints, mitigations, and opportunities for sustainable development of rice-based system in Laos

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Abstract— *Rice production in Xieng Ngeun District (XND), Lao People's Democratic Republic (PDR) faces several challenges that have resulted in a decline in rice production and a, therefore, food insecurity in the country. Smallholder farmers in XND, Luang Prabang in the Northern part of Lao are the most affected resulting in poor households and economies. Therefore, this study aims first to identify the key constraints affecting rice production systems; secondly to review the opportunities that exist in the rice production systems and lastly, to discuss opportunities that exist if the constraints are mitigated that can boost sustainable development of rice-based systems (SDRBS). A structured questionnaire was administered to village headmen, community members, and farmers in the nine selected villages with a total sample size of 374 farmers. Farmers' strength for rice is that they have agricultural land, on average 3 hectares per family. Apart from rice production, the majority of farmers have diversified into maize, Job's tears, vegetable cultivation and livestock keeping. The key strength of the respondent was the availability of land as most of them had more than 3ha of agricultural land. The diversification into the production of other crops is an important constraint to rice production. Labour was also an important constraint to rice production as most families have more than six children; thus, one member has to stay behind to take care of the children. The opportunities to improve rice production is mainly in the adoption of modern farming approaches using improved rice varieties, fertilizer application, use of herbicides for weed control and pesticides for insects and disease control. The farmers do not use pesticides to control insect pest and disease, and thus perennially faced challenges in rice production due to pest and diseases outbreaks. Moreover, there was a low rice productivity since majority of the the farmers did not use fertilizers in their fields, with a paltry 0.8% of the farmers recording positive response to fertilizer use. Despite this, most of the farmers (78.3%) indicated that they do not use herbicides for weed control. Market accessibility was a major concern to the farmers due to poor road infrastructure, hence resulting in delayed farm operations and produce spoilage. The farmers should be encouraged to adopt modern farming practices such use of pesticides to insect and disease management, weed control using herbicides for weed control, and fertilizer applications for improved soil fertility and yield. Moreover, there exist additional opportunities to improve rice productivity through adoption of improved rice cultivars, farm mechanizations, provision of extension services and improved market accessibility.*

Keywords— Rice production, Constraints, opportunities, Social-Economic, Luang Prabang, Laos.

I. INTRODUCTION

Rice is one of the most important food crops in the world after wheat [1] and a staple food for an estimated 3.5 billion people worldwide, especially in many developing countries [2]. Rice is currently grown in over a hundred countries that produce more than 715 million tons of paddy rice annually equivalent to 480 million tons of milled rice [3]. About four-fifths of global milled rice is produced by small-scale farmers in developing countries with almost a billion households in these regions depending on rice systems for their primary source of employment and livelihood [2].

The Per capita daily rice consumption in most Asian countries is the higher compared to other continents. Bangladesh, Cambodia, Indonesia, Myanmar, the Lao People's Democratic Republic (Lao PDR), Vietnam, Thailand, and the Philippines boasts of highest per capita daily rice consumption with reported intakes of over 300g per capita annually [3]. Rice is the staple food in Laos being grown on more than 49% of the cultivated land with annual production of about 3.27 Million tons of paddy mainly for subsistence consumption [4]. With a per capita consumption of milled rice per annum of 171 kilograms that constitute almost 70% of calorie and protein intake [4](Maclean *et al.*, 2002), rice production plays an integral part socio-economic lives of Lao's smallholders.

The three major rice production systems in Lao are paddy rice (season rice), irrigated rice and upland rice. More than 84% of production happens in the wet season under rainfed conditions [5]. While irrigated rice is cultivated along the Mekong River in the dry season. The lowland rainfed rice accounts for more than 70% of rice cultivated and produced in Laos with less than of 13% of total rice production occurring under irrigation conditions. Upland rice, on the other hand, is produced on steep slopes at higher altitudes above sea level up to as high as 1500 m [6], and is characterized by shifting cultivation and low productivity. No current data on extend to which upland rice is produced in Laos PDR is available, but Chazee (1994), estimated that about 2.1 million ha was used for rice production on a rotational basis under the 'slash-and-burn' cultivation system. More recently [7], estimated that area under upland rice cultivation had reduced to about 1.2 hectares in 2012 with a production of about 2.2 million tons.

The northern parts of Laos PDR is characterized by a large number of smallholder and resource-poor farmers that produce upland rice in sloping, unbounded fields under slash-and-burn systems without fertilizer under rainfed conditions for subsistence consumption[8][9]. This traditional upland rice cropping system accounts for more than 50% of the total rice produced in this region. With annual consumption of about 220 kg /person, the produced rice is sufficient for only 6-7 months. Despite effort and achievements of the Laos PDR government to ensure self-sufficiency of rice at the national level, various studies have reported that about 30% of the population has insufficient food for more than six months of the year [10][6].

1.1 Statement of the Problem

Annual rice production is unstable since most production is under rain-fed conditions accounting for over 10% of the annual variability in rice production. According to IRRI 1999, 87% of the rice produced in Laos in 1998, was rainfed and only 13% was produced under irrigation. There is need to sustainably reduce the area of rainfed upland rice and increase that of irrigated rice. There is still a significant yield gap in irrigated rice production that can be bridged without further investment in land and water development and increase rice production substantially

Almost all rice in Laos is transplanted by hand and harvested by no mechanized methods which hamper rapid and massive increase in yield. This system is labour intensive and hence increases the cost of farm inputs. More so hand transplantation is slow, and the cultivated rice most often is not uniform when the area under cultivation is large causing staggered harvest. Technological, productivity and climatic constraints such, unavailability or high cost of labor, unavailability of quality seed in time, unavailability of sufficient farmyard manure limit production of rice. Economic factors like price fluctuation and existing gap between rice grain and parboiled rice adversely affect rice production. The scarcity of land and water resources, environmental degradation, and loss of biodiversity had significantly limited the expansion of rice production in both developed and developing countries.

As the population and income of people are rising, the demand for food is increasing and meeting the food requirements of the growing population for global food security poses a considerable challenge. Growing prosperity is accompanied by human diets that will claim more natural resources per capita. This reality, combined with growing populations, may raise the global demand for food crops two- to four-folds within two generations. Deccline in land and water resources, environmental degradation, and loss of biodiversity limits development of sustainable food production in both developed and developing countries.

Biotic and abiotic constraints at farm level most significantly droughts and floods, poor soil fertility, pests and diseases and off-farm constraints such as high production costs, fluctuating market prices, and uncertain trade policy limit farmers' production beyond household self-sufficiency [5]. More so, increased intensity of cropping rice leading to low nitrogen and phosphorus in the soil coupled with inherent low water retention capacity of soils in this region against a background of irregular rainfall only serve to exacerbate the problem [9]. To ensure enough supply for the year, additional rice has to be procured from neighboring provinces. However poor road network coupled with the fact that poor smallholder farmers lack funds to frequently buy rice make this avenue unfeasible.

Sudden changes in rice policies in Laos are frequent and cause significant losses to both millers and farmers. For instance, Lao government policy in 2010 to liberalize the rice industry and allow farmers to export rice outside the country resulted in unexpected rapid exhaustion of rice stock forcing the government to import rice at very high prices [11]. Shifting cultivation has been associated with a decline in land and water resources, environmental degradation, and loss of biodiversity that

consequently limits the development of sustainable food production in both developed and developing countries. Constraints such as a dietary preference for rice and the inability to introduce tillage technology on sloping lands in Luang Prabang, however, make it hard to modify livelihood strategies and land-use [12].

Currently, no sustainable rice production systems that are easy to adopt which will help smallholder farmers exist in Xieng Nguen District (XND) and hence farmers are unable to produce enough rice for consumption and commercial purposes. The need to develop modern rice farming systems for resource-poor farmers of Xieng Nguen District is therefore apparent.

This study aims to identify critical constraints affecting rice production systems and review opportunities that exist in the rice production systems in XND, Lao PDR. The study also aims at designing complementary initiatives such as micro-insurance geared towards providing security to farmers in the face of unforeseen weather events. The findings of this study will help in establishing new systems that are sustainable, environmentally friendly, flexible and resilient to climate change.

II. MATERIAL AND METHOD

2.1 Study site location and description

The study was conducted in the uplands of northern Laos in Xieng Ngeun District (XND); about 25 kilometers to the south of the capital of Luang Prabang Province, on the Nam Khan River and Route 13, the main road to Vientiane (Figure 1). The study site was selected for this research based on the recommendation of the District Agriculture and Forestry Office (DAFO), and because it captured the fundamental research question that underpins this study.

Xieng Ngeun District has 49 villages, 6,600 households, and a population of 33,395 people. The villages included in the survey sample (Table 1) indicate the broad distribution of villages and the location of survey villages in this study. The key informants in this study were headmen, community members, and farmers from the nine (9) selected villages. Questionnaires were administered and the interviews conducted with the current farmers in the study area. A questionnaire, open-ended questions, was floated and same questions asked to individual respondents during the interviews.

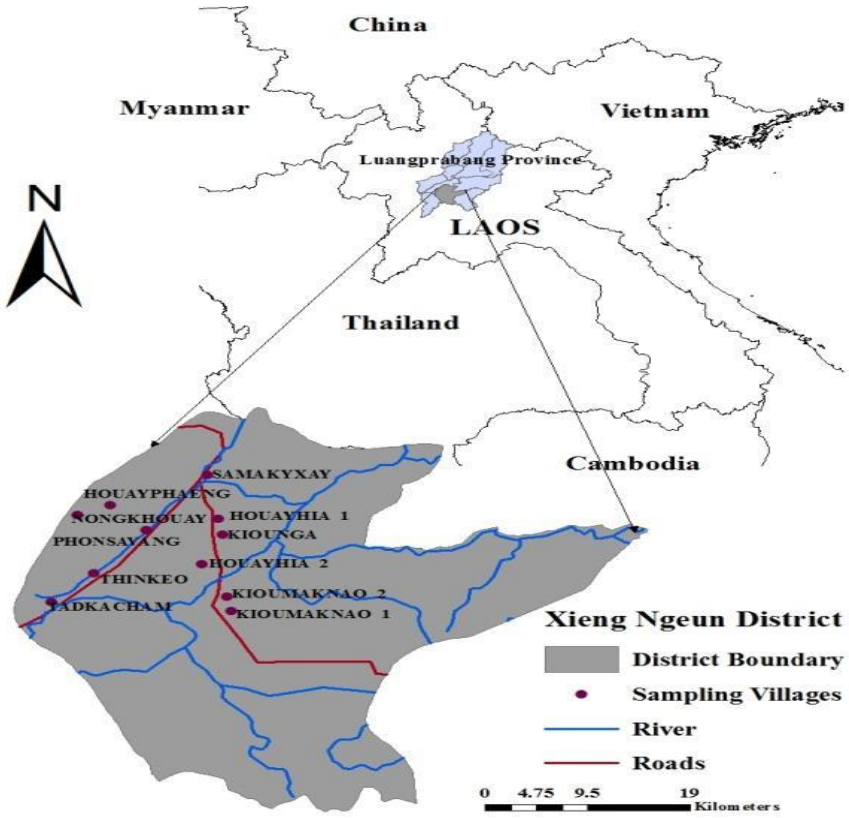

FIGURE 1: location of the study site and the villages sampled for the study. Source: Drawn by researcher, 2017

TABLE 1
LIST OF THE VILLAGES SAMPLED FOR SURVEY IN XND, LPB, IN LAO PDR IN 2017

Village Name	No. of households	Population per Village	NHH selected	Date of survey
Kioumaknao	150	843	57	9/2/2017
Kiouya	127	734	33	10/2/2017
Houy hear	124	721	43	11/2/2017
Houypheng	49	251	22	11/2/2017
Nongkuay	48	385	25	12/2/2017
Tadkacham	223	1,379	51	14/2/2017
Thinkeo	170	966	53	15/2/2017
Phonsavang	144	785	35	18/2/2017
Samackhyxay	655	3,419	55	20/2/2017
Total	**1,690**	**9,483**	**374**	

Source: by researcher (2017); NHH = Numbers of Households

Primary data were collected by interviewing individual farmers, groups of farmers, and government officials at the district and village levels. Structured and semi-structured interviewing techniques were adopted. Secondary data were gathered from a review of documents from several sources such as the Province Agriculture and Forestry Office (PAFO) and District Agriculture and Forestry Office (DAFO).

2.2 Rice Production Systems in the study area

The general information of rice cultivation in the nine study area villages in XND are shown below (Table 2). Rice production was the most common and significant economic, agricultural activity among the residents of the study area. Due to the mountainous nature of the study area, most of the households (74.4%) were engaged in upland rice production compared to paddy rice production. Upland rice production was significantly predominant in Kiouya village where all the household had an area of land set aside for rice production (Table 2). For smallholders who rely on a specific crop such as rice for their livelihood, it is, therefore, important to determine constraints affecting rice production systems to improve rice yield in the future. This calls for the involvement of key stakeholders to improve the farmer's livelihoods and improved food security.

TABLE 2
THE DISTRIBUTION OF THE FARMERS THAT ARE ENGAGED IN RICE PRODUCTION WITHIN THE SELECTED NINE VILLAGES, IN XND, IN LAO PDR IN 2017

Village name	NH	SpS	PP	NF	FUR	FUR (%)	FPR	FPR (%)
Kioumaknao	150	57	843	443	80	53.33	-	0
Kiouya	127	33	734	368	127	100.00	-	0
Houayhia	124	43	721	365	114	91.94	-	0
Houayphaeng	49	22	251	119	44	89.80	11	4.38
Nongkouay	48	25	385	184	46	95.83	3	0.78
Tadkacham	223	51	1,379	695	163	73.09	33	2.39
Tinkeo	170	53	966	454	64	37.65	49	5.07
Phonsavang	144	35	785	324	52	36.11	32	4.08
Samakeyxay	655	55	3,419	1,604	601	9		
Total	**1690**	**374**	**9483**	**4556**	**1291**	**74.39**	**128**	**1.86**

Source: A survey in 2017 by the researcher. Mark: NH=Number of Households; SpS = Sampling Site; NF = Number of Females; PP = Population, FUR = Families with Upland rice; FPR = Families with Paddy rice.

2.3 Climatic/weather pattern of Luang Prabang area

Luang Prabang is located in the mountainous area of Lao PDR. The area is characterized by high humidity and relatively high rainfall amounts. The rainfall is well distributed throughout the year, although with intermittent pockets of dry periods. The average annual temperatures range between 25°C and 26°C. Theoretically, these climatic characteristics are favourable for crop production throughout the year. Thus the study area is well suited for agricultural activities, including rice production among other agricultural activities. However, the study revealed that these favourable conditions are also suitable for weed growth and pest and disease infestations. Therefore, despite the favourable weather conditions for crop production, a slow increase in rice production has been recorded in the study area.

TABLE 3
THE CLIMATIC CONDITIONS OF LUANG PRABANG PROVINCE, LAO PDR BETWEEN THE YEAR 2010 AND 2015

Item/Year	2010	2011	2012	2013	2014	2015
Rainfall	1368.6	2233.5	1259.4	1747.6	1468.5	1525.1
Temperature	27.1	25.8	27.0	26.3	26.5	26.6
Humidity	72.0	75.0	74.5	79.5	71.5	60.5
Sunshine	2,277.5	1,920.3	1,923.8	1,880.9	2,013.6	1,957.1

Mark: The units are annual amounts in; Rainfall (mm), Temperature (°C), Relative Humidity (g/m³)

2.4 Analysis of Qualitative and Quantitative Data

The sample size of 374 respondents, comprising of headmen, farmers, groups of farmers, and government officials was selected for the study. This sample size allowed for statistical analysis with confidence. Descriptive statistics were used to analyze quantitative data with social science statistical software (SPSS). Descriptive statistics provided a method to describe trends and draw generalisations and conclusions about the farmers using statistically significant results. An exploratory factor analysis was conducted on the frequency statements as many significant correlations were found within the data. Both quantitative data and qualitative are reflections of the process that provided the findings. Identification of constraints and opportunities for the sustainable development of rice production systems in XND was based on observations of the study area by interviewers, and collection of secondary data from any source on important agricultural factors such as environmental temperature, amount of rainfall and distribution, occurrences and frequencies of floods and natural disasters, incidences of insect pests and diseases, and general social-economic status of the respondents.

III. FINDINGS DATA ANALYSIS AND RESULTS

3.1 Characterization of the Socioeconomic Status of the Respondents

The age of the respondents ranged from 18 years to 90 years, with majority of the respondents, both males and females ranging between 28-37 years of age. Interestingly, the youngest farm owners ranged between 18 – 27 years of age. This finding observes that a good proportion of the younger generations are venturing into agricultural farming. It brings decisive significance as they can adopt new and necessary innovations in the farming fields. Most of the respondents (55.6%) were male compared to the 44.6% female respondents. Majority of the respondents (87.7%) were from Kummou ethnic group. The number of women ranged from zero to 11, and with a maximum of eight (80 children per household. Generally, the study area was characterized with large size of household member, the maximum being seventeen (17) members and a minimum of 2.

TABLE 4
SOCIOECONOMIC CHARACTERIZATION OF THE RESPONDENTS

Variable	Mean ± S.E	Std. Deviation	Minimum	Maximum
Age	42.0 ± 0.69	13.25	18	90
Number of Children	1.54 ± 0.081	1.53	0	8
Number of Women	3.00 ± 0.08	1.60	0	11
HH member	6.14 ± 0.12	2.36	2	17
Bicycle	0.26 ± 0.029	0.56	0	3
Motor bike	1.25 ± 0.050	0.96	0	6
Car/ Truck	0.06 ± 0.01	0.25	0	2
Hand tractor	0.04 ± 0.01	0.20	0	1
TV	0.87 ± 0.02	0.43	0	3
Radio	0.17 ± 0.02	0.39	0	2
Rice Mill	0.26 ± 0.02	0.44	0	1
Refrigerators	0.51 ± 0.03	0.53	0	2
Cell phone	2.69 ± 0.09	1.64	0	13
Bank Account	1.71 ± 0.02	0.38	1	2

Source: Survey by researcher, 2017

The mean age of the respondents was 42.0±0.69 years with a standard deviation of 13.25 and was statistically significant as the t-value was smaller than the difference freedom (*df = 373*). Again, the standard deviation indicated a small deviation

from the main population means resulting in a good representation of the mean. The descriptive analysis showed that the respondents' households had more children than women. Majority of the respondents owned either bicycles or motorbikes that they used as transportation to the farms or the markets for their daily activities (Table 4). Again, most of the respondent farmers had hand tractors for their ploughing. Most of the respondents also owned devices such as cell phones, radios and television sets, either for communication or entertainment purposes respectively. Nonetheless, there was a greater number of televisions, refrigerators and cell phones.

These findings illustrate that the respondents had more access to information on various agricultural techniques and equipment. Also, the mean for the number of bank accounts by the farmers was high indicating a statistical significance and a t-value lower than the difference freedom. The respondents also had a great number of speakers and DVDs that could be used for entertainments. All the descriptive socioeconomic variables were significant at 95% confidence level. It could be supported by the *sig. 0.000*** that indicates that the values fall under 0.0005, *(0.0005< p>0.05)*. Again, large households are proxy of labor endowment that would enable them to accomplish various farm tasks on a timely basis. In essence, it could increase the farm rice productivity.

3.2 Landholding per farmer (How many hectares)

All the respondents interviewed during the survey managed a piece (s) of land where they practiced various agricultural activities (Table 5). The sizes of land varied significantly from 1 hectare to 16 hectares. The possibility of managing land did not necessarily translate to land ownership. Majority of the farmers did not have their pieces of land, but instead either leased from other farmers or households. Thus there was a strong correlation between the land ownership to the types of crops or livestock the responded kept in the farm. There was a mixed aspect to land utilization from one village to another and from individual farms to another. In a simple frequency analysis, most (78%) farmers observed that the available land parcels are further subdivided into smaller units that can contain intercropping and livestock rearing. It is a challenge as large farms of rice would lead to higher yields of product.

TABLE 5
LAND SUBDIVISION CHARACTERIZATION WITHIN THE STUDY AREAS IN XND, LPB, LAO PDR IN 2017

Land area (hectare)	Mean ± S.E	Std. Deviation	Minimum	Maximum
Total number of parcels managed	4.564 ± 0.128	2.482	1.0	16.0
Land Area (hectare)	4.330 ± 0.185	3.577	0	33
Number of parcels for Agriculture	3.265 ± 0.108	2.088	0	11.0
Agricultural Land (Hectare)	3.601 ± 0.155	2.995	1.0	2.0

Source: Survey by research, 2017

The characteristic values for the land size and partitioning amongst the respondent farmers are illustrated above (Table 5). Most of the farmers managed between 3 and 4 pieces of land. It was observable that some of the respondents (10.4%) also had no pieces of land to maintain. The highest number of managed parcels was 16. The overall mean for the land management was statistically significant with a standard deviation of 2.482. On the other hand, the land area representation had a mean of 4.33 ± 0.19 and a variable deviation of 3.58. The significant level was statistically correct as the p-value was less than the recommended threshold. Most of the respondents had between 0.1- 3 hectares of agricultural land. It illustrated that most of the respondents are small-scale farmers with just a few (2.7%) being large-scale farmers. The findings indicated that most of the land pieces engaged in agricultural activities.

TABLE 6
CROP DIVERSIFICATION IN TERMS OF TOTAL LAND AREA AND CORRESPONDING YIELD PER HECTARE PER CROP AMONG THE INTERVIEWED FARMERS IN XND, LPB IN 2017.

Type of crop	Area (hectare) *	Yield (tons) *	Rice yield (tons)	
			Minimum	Maximum
Upland rice	0.687±0.034	1.248±0.064	0.0	5.2
Paddy rice	0.090±0.014	0.317±0.051	0.0	7.5
Maize	0.176±0.018	0.670±0.071	0.0	6.0
Job's tears	0.225±023	0.793±0.083	0.0	10.0
Banana area	0.026±0.008	0.012±0.007	0.0	2.0
Fallow	1.662±0.090	-	-	-

****The values within the columns marked with an asterisk (*) represent the mean ± the standard error of the mean (S.E) for the individual category. The minimum value represents the minimum amount/Quantity of the product per respondent.***

Majority of the respondents (79%) had rice plantations, with the majority of the responded cultivating upland rice type compared to paddy rice. A chi-square indicated a statistical significance between the two groups of rice types. The findings also showed that the upland rice productivity was higher and significant at 10% confidence level. Due to the mountainous nature of the study area, upland rice production is the most feasible of the two rice production systems. Moreover, the environmental characteristics of the study area favours upland rice production compares to paddy rice.

Maize production was higher than paddy rice production as the mean of maize production in tons was higher than the paddy rice production mean, (0.670±0.071 > 0.317±0.051). Other land crop partitioning included the cultivation of bananas and fallow land. A more significant chunk of land parcels was not ploughed in the study area. Again, the findings observed that Job's tears was the second highly cultivated crop in the study area. It contained a statistical mean of 1.82. In cumulative, rice formed the highest produced crop within the study area justifying the significance of researching the constraints affecting its production.

3.3 Agricultural land in the study area

Most residents of Luang Prabang province practice rotational farming system. This is characterized by farming in one parcel of land for 3-5 years, followed by relocation to a new piece of land. This system is apparently evident and is represented by the data that reflects constant changes in the total area under rice production. Generally, the area under rice production has been on the increase, with few instances of decline in total area under production. The increase punctuated with a few cases of a decrease in total area of land did not result in a significant change in the total rice production within the province.

TABLE 7
TOTAL LAND AREA (HA) UNDER RICE PRODUCTION IN LPB BETWEEN 2010 AND 2015

Rice Type/Year	2010	2011	2012	2013	2014	2015
Season Rice	627,865	598,358	706,028	683,125	739,932	769,193
Irrigated Rice	108,410	112,210	107,967	92,340	102,504	99,019
Upland Rice	118,839	106,682	119,772	115,725	115,400	116,720
Total	**855,114**	**817,250**	**933,767**	**891,190**	**957,836**	**984,932**

There has been a general increase in rice production in Luang Prabang province. This is exemplified by a steady increase in the total quantity (tons) of the rice produced from the year 2013 to 2015. It is also interesting to note that there were years when there was a nonsignificant decline in total rice productivity. The season rice/wet season rice is the leading source of rice in Lao. The season has been contributing to a significant amount of rice from the year 2006 to the time of the study. There has been a significant contribution in the total rice production from upland rice production system. This can be explained by the opening up of new agricultural lands in the formerly fallow upland regions. Moreover, the development of new rice cultivars specifically bred for upland production systems could have contributed to the increased contribution in total production from upland rice.

TABLE 8
GENERAL TREND IN RICE PRODUCTION (TON) PER UNIT AREA IN LPB BETWEEN 2010 AND 2015

Rice type/Year	2010	2011	2012	2013	2014	2015
Season Rice	2,331,330	2,323,195	2,763,150	2,734,970	3,211,584	3,357,640
Irrigated Rice	512,430	540,315	509,920	439,150	555,086	520,000
Upland Rice	226,880	202,250	216,140	240,440	235,755	224,360
Total	**3,070,640**	**3,065,760**	**3,489,210**	**3,414,560**	**4,002,425**	**4,102,000**

The study reveals the existence of a positive correlation between the total rice production (tons) and the total area under rice production. There was a general increase in rice production with the corresponding increase in total area under rice production. This is an expected phenomenon especially when all other factors of production are kept ceteris Paribas. However, the increase in rice production was not unit by unit proportional to the increase in total area under rice production.

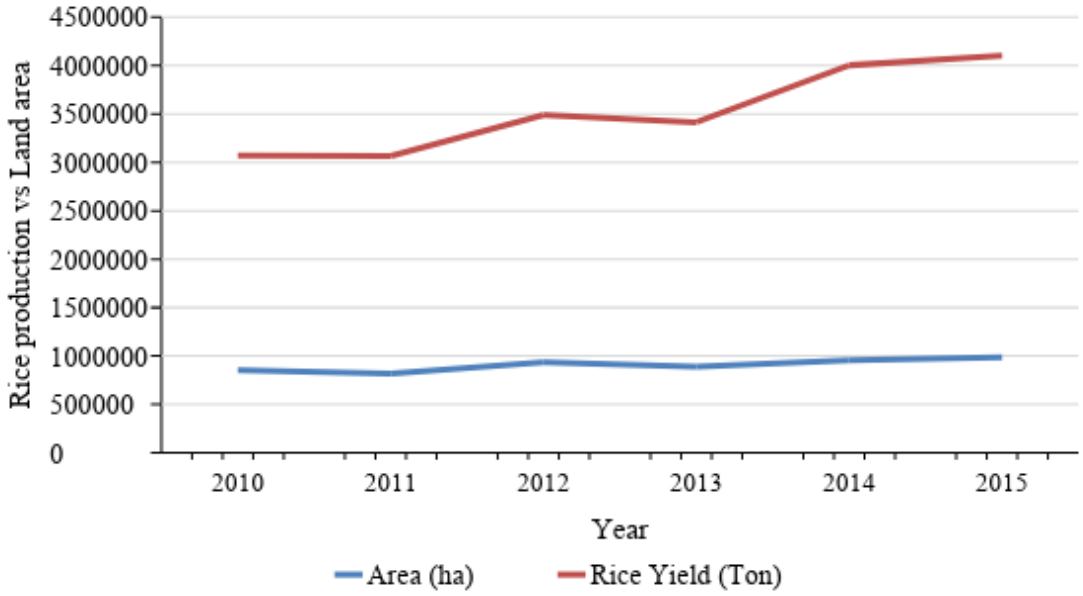

FIGURE 2. Comparative between rice production increasing with area increasing, in 2010 to 2015

Despite there being a steady increase in the total area under rice production, the increase was not statistically significant. As a result, there were seasons that the total increase in rice production was not proportional to an increase or decline in the total area under rice production. Rice production also varied from one study village to another. This mostly depended on whether the village produced upland or paddy rice. The upland rice was mainly produced in the higher mountainous regions of the study area. Thus, paddy rice production was not recorded in the mountainous villages such as Kioumaknao, Kiouya, Houayhia and Samakeyxay.

TABLE 9

RICE PRODUCTION STATISTIC IN THE SELECTED STUDY VILLAGES

Village Name	Upr (Ha)	Upr (Ton)	Pdr (Ha)	Pdr (Ton)
Kioumaknao	97.00	135.80	-	-
Kiouya	138.00	193.20	-	-
Houayhia	120.00	168.00	-	-
Houayphaeng	54.00	75.60	7.70	23.10
Nongkouay	45.70	63.89	1.60	5.50
Tadkacham	180.47	252.66	27.70	171.30
Tinkeo	64.62	129.24	28.55	164.40
Phonsavang	52.50	125.00	16.80	58.80
Samakeyxay	590.50	885.00	-	-

Mark: Upr = Upland rice; Pdr = Paddy rice

TABLE 10

PROPORTION OF THE RESPONDENTS THAT PRACTICE CROP PROTECTION AND FERTILIZER USE AMONG THE RESPONDENTS IN XND, LPB, LAO PDR IN 2017.

Parameter	Proportion (%)	Quantity (litres or kg) (AQY(L)	Minimum (L/kg)	Maximum (L/Kg)
Herbicide use for weed control (HWC)	21.7	1.51 ± 0.21	0.0	40.0
Pesticide use for pest control (DPC)	0.00	0.0	0.0	0.0
Fertilizer application (DFC)	0.80	0.508 ± 0.31	0.0	90.0

***Source: Survey 2017. Mark: HWC = Herbicides use for weed control (1 No; 2 Yes); AQY = Approx. Quantity of use per year (L); DPC = did you use Pesticides for crop (1 No; 2 Yes); DFC =* did you use fertilizers for crop (1 No; 2 Yes)**

Table 10 illustrates the crop protection and management mechanisms within the study area. It focused on the substances such as herbicides, fertilizers, and pesticides. The findings observed that the respondents preferred none of the elements. Most of the respondents (78.3%) indicated that they do not use herbicides to control weeds in their rice farms. On the other hand, only 21.7% had used herbicides for weed control in rice fields. Among the respondents who used the herbicides, majority used between 5 -10 liters. On the fertilizer, only 0.8% of the respondents acknowledged using fertilizer with the most substantial number of farmer respondents not using pesticides summing up to 99.2 %. Among the respondents who used fertilizer, they preferred 90kgs annually. Finally, no respondent had used pesticides in their rice farms. There were more farmers using herbicides compared to fertilizers and pesticides. Therefore, it is observable from the findings that one of the problems that could be affecting rice production in XND was lack of fertilizer application and pest management.

TABLE 11
THE PROPORTION OF RESPONDENTS THAT WERE ENGAGED IN LIVESTOCK REARING IN XND, LPB, LAO PDR IN 2017.

Variables	Proportion (%)	Mean ± S.E	Minimum	Maximum
Buffalo	5.10	0.184 ± 0.052	0.0	10.0
Cows	27.00	1.757 ± 0.294	0.0	61.0
Pigs	63.60	3.267 ± 0.239	0.0	38.0
Goats	24.10	1.525 ± 0.194	0.0	25.0
Chicken	73.30	18.957 ± 1.621	0.0	300.0
Duck	29.10	2.511 ± 0.318	0.0	50.0

Source: Survey by researcher, 2017

Among the respondents, 88.2% engaged in livestock production leaving 11.8% as having no livestock. A more significant number, 94.9% had no buffalos with the modal number being 1 – 3 buffalos. However, the number of buffalos was statistically correlated to the number of the farmers who had more than six pieces of land to manage. It is, therefore, observable that Buffalo rearing was practiced purposely for ploughing the land, as the large number and size of the lands would not be easily ploughed using hand tools. It was also found that most of the farmers had no cows, representing 73% of the total respondents. Nonetheless, the majority of the respondents (63.6%) engaged in pig production. Very few percentage was slotted for goat rearing with the individuals not having goats represented by 75.9%. Interestingly, 73.3% of the respondents had chickens in their households. Not many (29.1%) engaged in duck production. It is observable that the respondents do not participate in mixed farming and the preferred kind of animal was a pig. The variables contained a small variation margin from the means indicating the compactness of the variable distribution. Again, the variables were statistically significant at 95% confidence level.

TABLE 12A
DISTRIBUTION OF FARMERS' SOURCES OF INCOME (US$) IN XND, LPB, LAO PDR IN 2017

Income category	Mean ± S.E	Std. Deviation	Minimum	Maximum
Total Household Income	2698.16 ± 135.79	2626.00	55.00	16500.00
Government or Public service (US$)	484.06 ± 70.99	1370.98	0.00	11250.00
Wages, Temporary employment	814.97 ± 81.19	1570.23	0.00	15125.00
Trade incl. small shops or business (US$)	212.60 ± 44.39	858.43	0.00	6875.00

Source: Survey by researcher, 2017

The findings indicated that most of the respondents enjoyed a house income in the range of 55 – 2000. Few respondents, 2.4% had a household income higher than 10,000. The finding depicts that most of the respondents are either small-scale farmers or work on various farms owned by others. They were purely farmers with no other occupation. Averagely, the earnings from either the public or government were statistically significant and displayed a small deviation from its mean. It was observed that 43.6% of the income earned was as a result of temporary employment. Therefore, the majority of the respondents who had other jobs apart from farming were temporarily employed. It was distributed to the right with a skewness of 1.76. The majority, 90.6% did not engage in trade either in small shops or large businesses.

TABLE 12B
PROPORTION OF FARMERS' INCOME (US$) DISTRIBUTION FROM SALE OF AGRICULTURAL COMMODITIES AND OTHER ACTIVITIES IN XND, LPB IN LAO PDR IN THE YEAR 2017

Income Activity	Mean ± S.E	Std. Deviation	Minimum	Maximum
Sale of Rice	66.93 ± 8.85	171.11	0.00	1162.5
Sale of other agricultural crops	517.75 ± 46.95	907.94	0.00	12375.0
Sale of non-timber forest products	103.06 ± 11.78	227.76	0.00	2500.0
Sale of livestock	369.93 ± 45.37	877.51	0.00	9375.0
Sale of Fish	0.836 ± **0**.69	13.32	0.00	250.0
Sale of handicrafts	16.862 ± 4.27	82.52	0.00	875.0
Money sent home, remittance	120.06 ± 23.34	451.46	0.00	6250.0

Source: Survey by researcher, 2017

Farmers received more revenues from the sale of other agricultural products as compared to the sale of rice. About 81.8% did not participate in rice sales, indicating that most of the farmers produce rice for their domestic consumptions leaving 18.2% for trading. However, other crops such as bananas sold heavily in the markets. It displayed a 71.1% positivity in sales revenue, and only 26.7% of the farmers never received revenues from the other crops. Again, few farmers (36.4%) engaged in the sale of non-timber forest products. A more considerable portion of the livestock sale was significant. Regarding the market share, both the sale of livestock and other agricultural products had the highest demands displayed from their level of revenue turnover. Few or no farmers (99.5%) engaged in fish production sales within the villages where the study was conducted. A few farmers (6.7%) also engaged in the sale of handicrafts as an alternative earning projects. About 17.1% participated in remittances after sales with a higher number of the respondents not being able to send money back home. However, amongst those who attended in payments, the greater number lied between the range of 1 – 1000 (US$).

TABLE 13
TEST FOR MULTICOLLINEARITY FOR HOUSEHOLD CHARACTERISTICS AND SOCIO-ECONOMIC STATUS OF THE RESPONDENTS IN 2017

Model	Collinearity Statistics	VIF
	Tolerance	
(Constant)		
Age	.875	1.143
Sex	.885	1.131
Ethnic	.768	1.302
Number of women	.701	1.427
Number of Children	.673	1.486
Have you always lived in this village	.830	1.204
Bicycle	.906	1.104
Moto bike	.777	1.287
Car/truck	.893	1.119
Hand tractor	.856	1.169
TV	.792	1.262
Radio	.862	1.160
Rice mill	.730	1.370
Refrigerator	.600	1.667
Cell phone	.720	1.390
Bank account (1 No; 2 Yes)	.824	1.214
VCD/DVD	.822	1.216
Speakers	.770	1.299
Total number of pieces of land managed per respondent	.755	1.325

Source: Survey by researcher, 2017

Variance Inflation Factor (VIF) and Pearson correlation matrix test methods were employed to test for the multicollinearity among the explanatory variables. VIF test was carried out by determining 'artificial' Ordinary Least Squares regressions where every independent variable was regressed against the rest of the explanatory variables. The results show that VIF of all the independent variables were below 2.00 (Table 13). As a rule of thumb, if the VIF is greater than 5, then the variable is said to be highly collinear [13]. Pearson's correlation matrix test also indicated that all the correlations between the

independent variables were below 40 percent, reaffirming non-existence of multicollinearity in the data. Following [13], explanatory variables with correlations less than 75 percent are considered to have autocorrelation.

IV. DISCUSSIONS

This study focused on the constraints and opportunities for sustainable development of rice production system in XND, Luang Prabang province in Laos. The study sought to identify the challenges and provides potential mitigation of the problems to enable sustainable rice production in the said area. Over the past year, rice production in Lao has increased a lot, as the country has achieved self-sufficiency of food. Despite the high rice productions in the country, this agricultural sector has had its equal share of opportunities and challenges.

The poor road infrastructure within the study area was a great hindrance to the transportation of agricultural commodities to the market. The poor road conditions also affected rice production activities as the farmers would take longer time to access their farms on foot. This means that the good part of the time is spent on walking to the farms rather than engaging in productive agricultural activities, especially rice production. There is a likelihood of a substantial amount of loss of agricultural produce through post-harvest losses and handling of the produce. This can also be associated with the poor road infrastructure that results in either delayed delivery of the produce to the storage facilities or spillage of the produce on transit. The poor road network could, however, be explained by the hilly, rugged and rough terrain characteristic of the study area and a larger part of Lao PDR.

Land fragmentation has been blamed for a decline in agricultural productivity and potential food insecurity in many developing countries [14]. Most of the respondents had several pieces of land that they either individually owned or leased from other households. A good number of this mentioned parcel of land were lying fallow for several seasons, either to regain their reduced fertility or due to the inability of the farmer to cultivate it. This resulted in a significant number of the agricultural land area being recorded but without a corresponding increase in yield. The quantity of the returns from the small parcels is not comparable to the amounts that would be achieved from the large parcels of land. Increasing the size of the farming parcels results in a positive productivity coefficient. Moreover, the relationship between land size and rice production can be as a consequence of returns to scale. However, several studies have suggested that the return to scale is merely a constant. Again, since the shadow prices of factors of production change with changes in the size of land holding, farmers are forced to apply more of the elements to which they access quickly and the ones that command lower shadow prices. Therefore, it is expected that the above explanation can be applied in the case of XND region and that the marginal productivity analysis is at constant levels. Finally, the principal differences in size of land parcels, as well as land management practices, might lead to the differences in the economic life of the rice farmers within the same locality.

4.1 Household sizes and lack of labour as a challenge

Lack of or inadequate labour supply/availability impacts negatively on agricultural productivity especially in non-mechanised farming systems. The analysis of the responses from the farmers indicated that they lacked enough farm labor despite the relatively large households. The large household sizes did not necessarily translate to the availability of farm labour. The large households were due to a higher number of children per family that were not within the legal working age limit. As a result, in most of these households, one of the adult members was tasked with the duty of taking care of the children, thus cutting further on the available labour force in the family. In addition, the lure of better employment opportunities also confounded the inadequate labour problem within the study region. Rural to urban migration has been a significant challenge in the availability of labour in many parts of the world [15][16], and continue to threaten food production and consequently food insecurity in many developing countries.

4.2 Irrigation system challenges

Continued reliance on rainfed farming activities has been pointed out as the potential source of decline in agricultural productivity and is seen as a threat to food security in many parts of the world. Rice production, especially paddy rice, requires an adequate water supply and for most of the growth period, flooding of the land. Smallholder farmers in Lao PDR consistently rely on rainfall for farming activities and rice production. In the cases of unpredicted droughts, massive crops failures had been reported in most parts of the country. In such instances, most farmers, especially those with parcels of land that border rivers/streams, had to resort to irrigation. The irrigation system is poorly developed and comes as an expensive last resort for most farmers. The farmers were grappling with unreliable rainfall and periods of dry spells in some of the areas. This was noted as a challenge among many farmers who did not have irrigation systems in place.

4.3 Diversification and income from sale of farm commodities

The uncertainties that exist in agricultural activities has driven many farmers into diversifying crop and animal production. The findings of the study highlight the high level of crop diversification among the respondents. This could be attributed to need to cushion themselves from uncertainties that could arise from one crop failure. Also, having an alternative source of income is a common characteristic of many smallholders. [17] Suggested that diversification of farming systems, land tenure and human capital formation by the government and multilateral development agencies would successfully enhance livelihood in Luang Prabang. This is in line with numerous past research studies that observed that needs of rural communities in the province are highly differentiated and require locally adapted self-sufficient, diverse, economically viable and small-scale agro-ecosystem based strategies.[18][19][20].

4.4 Sources of household income and remittances

Farmers received more revenues from the sale of other agricultural products as compared to the sale of rice. The income from the sale of rice among the respondents was low compared to other sources of income. This could be attributed to low rice productivity within the region. The smallholders characteristically practice subsistence farming and only sell the surplus produce, if any [21]. Among the residents of XND, only a small proportion of the population recorded income from government or public services. This could be an indication of lack of employment opportunities in the public sector. The lack of formal employment coupled with non-engagement in agricultural activities explains the low household income among the respondents. As a result, there was a small remittance after sales with a higher number of the respondents not being able to send money back home. This is an indication of the poor economic status of the respondent and that the little that they earned was only enough to sustain their families. The study was however not able to unravel where the bulk of the potential labour force is utilized.

4.5 Biotic and abiotic challenges to rice production

Insect pests and diseases have resulted in a decline in both quality and quantity of agricultural produce in many countries [22][23]. Biotic and abiotic constraints at farm level most significantly droughts and floods, poor soil fertility, pests and diseases and off-farm limitations such as high production costs, fluctuating market prices, and uncertain trade policy limit farmers' production beyond household self-sufficiency [5]. Several insects pests and diseases continuously attack rice in the field, during harversting period and/or in storage. The respondent pointed out that they were struggling with insect pests and diseases management on their farms. Astonishingly, most of the farmers did not practice use of pesticides to avert challenges due to these biotic stresses. This could be attributed to the poor economic status of the farmers and thus their inability to afford the chemicals. Therefore, despite the continued efforts put in rice production, there is a substantial amount that is lost due to pest and disease damage. Lack of pest control mechanisms among the respondents was a significant challenge to the rice farmers. From the descriptive analysis, most of the farmers if not all had not used any of the pesticides in to protect their crops. In most cases, herbicides and pesticides are used in controlling weeds rice insect pests, respectively

Continuous cultivation of one crop, or crops in the similar family, on the same piece of land results in a decline in soil fertility. Most cereal crops are heavy miners of nutrients and minerals from the soil. There is always a need to apply organic or inorganic fertilizers to maintain or improve the fertility status of land for continued productivity. Most farmers in Lao do not use fertilizers for crop production due to the perceived high fertility of the land. However, the decline in fertility in these lands has made most farmers resort to shifting cultivation, with slash-and-burn systems being prevalent. The practiced shifting cultivation does not allow for sufficient rest period for the land to regain natural fertility. In addition, the slash-and-burn system has been blamed for degradation in soil fertility and destruction of beneficial microorganisms in the soil [24] [25][26]. More so, increased intensity of cropping rice leading to low nitrogen and phosphorus in the soil coupled with inherent low water retention capacity of soils in this region against a background of irregular rainfall only serve to exacerbate the problem [9].

4.6 Access to agricultural information and extension services

Farmers across the globe are faced with farming uncertainties as a result of lack of agricultural information. Such information relates to farming inputs, pest and disease control, ploughing periods, as well as harvesting techniques. Differences in accessing various agricultural farming information are the reasons that have resulted in different crop productivity yields. However, the lack of response when the farmers were asked whether they get help from the government could be interpreted as lack of extension services, either because of the poor accessibility of the areas or for other reason (s) that was/were not captured during the study.

V. CONCLUSION

There are no sustainable rice production systems that are easy to adopt which will help smallholder farmers existing in XND, PLB, Lao PDR and hence farmers are unable to produce enough rice for consumption and commercial purposes. Smallholders in Lao PDR face several challenges in farming, especially in rice production. The major obstacles to rice production were lack of agricultural inputs, poor road infrastructure, lack of access to extension services, period drought and crop damages due to insect pests and diseases. To improve upland rice productivity, the irrigation systems must be implemented to assist the rice farmers. The farmers also need to be sensitized on the negative impacts of slash-and-burn systems on the soil fertility. Moreover, emphasis should be placed on the use of fertilizers and pesticides to improve soil fertility and to control rice pests and diseases respectively, in their farms. This call for the improvement of extension services to the farmers through the concerned government departments and ministries. To enhance this, the government need to invest in development of the road infrastructure. This will not only boost access to the villages but will also enable the farmers to transport farms inputs and produce to and from the farm respectively.

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REFERENCES

[1] Jones, Monty P. *The rice plant and its environment*. WARDA Training Guide 2. WARDA, Bouaké, 1995, p. 27-30.

[2] International Rice Research Institute. 2013. World Rice Statistics 2013. Los Banos, the Philippines: IRRI. June 29, 2013. *http://irri.org/index.php?option comk2& view= item&id=9081&Itemid=100481&lang=en. IRRI (2014). http://irri.org/news/hot-topics/rice-and-climate-change.*

[3] Food and Agricultural Organisation. 2013. FAOSTAT Database. Rome: Food and Agricultural Organization.

[4] MAF, 2012. Lao Census of Agriculture2010/11 Highlights.

[5] Schiller, J.M., Linquist, B., Douangsila, K., Inthapanya, P., Douang Boupha, B., Inthavong, S. and Sengxua, P. Constraints to Rice Production in Laos, 2001.

[6] ADB. 2001. Participatory Poverty Assessment in the Lao PDR. Manila.

[7] Khamko, T., Gang Hua, L.I., Edzesi, W. M. (2016). Constraints and Opportunities for the Sustainable Development of Rice Production Systems in Laos. International Journal of Business, Economics and Management Works. Kambohwell Publisher Enterprises ISSN: 2410-3500. Vol. 3, Issue 5, PP. 66-74, May 2016.

[8] Linquist, B.A., Trosch, K., Pandey, S., Phouynyavong, K., Guenat, D. Montane paddy rice: its development and effects on food security and livelihood activities of highland Lao farmers. Mountain Res. Dev. 27, 2007, p.40–47.

[9] Roder, W. Slash-and-Burn Rice Systems in the Hills of Northern Laos PDR: Description, Challenges and Opportunities. International Rice Research Institute, Los Baños, Philippines, 2001, p. 201

[10] United Nations' World Food Programme (UNWFP). (2005). World Hunger Laos. Available: http://www.wfp.org/country_brief/indexcountry.asp?country=418

[11] NURiFaR, 2012. Rice marker assessment in NURiFaR target provinces

[12] Roder, W. Slash-and-burn rice systems in transition: challenges for agricultural development in the hills of northern Laos. Mountain Research and Development, 1997, p.1–10.

[13] Gujarati, D.N. 2003. Basic Econometrics. New York: McGraw Hill Book Co. p328.

[14] Alemu, G. T., Ayele, Z.B. and Berhanu, A.A. (2017). Effects of Land Fragmentation on Productivity in Northwestern Ethiopia. *Advances in Agriculture*, vol. 2017, Article ID 4509605, 9 pages, 2017. doi:10.1155/2017/4509605

[15] Angba, A.O. (2003). Effect of rural-urban migration of youths on agricultural labour supply in Umuahia North Local

[16] Mbah, E.N., Ezeano, C.I. and Agada, M.O. (2016). Effects of rural-urban youth migration on farm families in Benue state, Nigeria. *Int. J. Agril. Res. Innov. & Tech*. 6 (1): 14-20.

[17] Bravo-Ureta, B., Solı´s, D., Cocchi, H. and Quiroga, R., 'The impact of soil conservation and output diversification on farm income in Central American hillside farming', *Agricultural Economics*, Vol. 35, (2006) pp. 267–276.

[18] Akram Lodhi AH. 2008.(Re)imagining agrarian relation. The World Development Report 2008: Agriculture for development *development and change* 39(6): 1145-1161.

[19] Thongmanivong S, Fujita Y. 2006. Recent land use and livelihood transitions in Northern Laos. *Mountain Research and Development* 26(3): 237-244.

[20] Wale, E. and Yalew, A. 2007. Farmers' Variety Attribute Preferences: Implication for Breeding Priority Settings and Agricultural Extension Policy in Ethiopia. African Development Bank, 379-396.

[21] Harvey, C. A., Rakotobe, Z. L., Rao, N. S., Dave, R., Razafimahatratra, H., Rabarijohn, R. H., MacKinnon, J. L. (2014). Extreme vulnerability of smallholder farmers to agricultural risks and climate change in Madagascar. Philosophical Transactions of the Royal Society B: Biological Sciences, 369(1639), 20130089.

[22] http://doi.org/10.1098/rstb.2013.0089.

[23] Kihoro, J., Bosco, N. J., Murage, H., Ateka, E., & Makihara, D. (2013). Investigating the impact of rice blast disease on the livelihood of the local farmers in greater Mwea region of Kenya. SpringerPlus, 2, 308.

[24] http://doi.org/10.1186/2193-1801-2-308.

[25] Cerda, R., Avelino, J., Gary, C., Tixier, P., Lechevallier, E., et al. (2017). Primary and Secondary Yield Losses Caused by Pests and Diseases: Assessment and Modeling in Coffee. PLOS ONE 12(1): e0169133.

[26] Adeniyi, S. A. (2010) . Effects of slash and burning on soil microbial diversity and abundance in the tropical rainforest ecosystem, Ondo State, Nigeria. *African Journal of Plant Science*, 4(9) 322 – 329.

[27] Beliveau, A., Davidson, R., Lucotte, M., Lopes, O.C., Paquet, S. and Vasseur, C. (2015). Early effects of slash-and-burn cultivation on soil physiochemical properties os small-scale farms in the Tapajos region, Brazilian Amazon. The Journal of Agricultural Science, 153(2) 205 – 221.

[28] Giardina, C. P., Sanford, R.L., Døckersmith, I.C. and Jaramillo, V.J. (2000). The effects of slash burning on ecosystem nutrient during the land preparation phase of shifting cultivation. *Plant and Soil*, 220: 247 – 260.

Survivability of *P. oxalicum* T3.3 bioformulation on carrier materials and storage temperature

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Abstract*—Good bioformulation play crucial roles in the successfully of commercialize biological control products. The development of bioformulation is necessary to improve product stability, delivery and bioactivity. The aim of this study is to assess the shelf life of P. oxalicum T3.3 conidia in the different ratio(1:1,1:2,2:1) of Biochar:Biocompost (BcBp) and Peat:Vermiculite (PtVm) and temperature (4ºC and room temperature) for 6 months. The results showed that P. oxalicum T3.3 was able to sustain highest viable cell (CFU) at 4ºC storage temperature. BcBp and PtVm have the highest cell viability at ratio 2:1 and 1:1, respectively. Both BcBp and PtVm showed potential carriers for the development of biofungicide for agriculture purposes.*

Keywords— Biochar, Formulation, Peat, Penicillium, Shelf life.

I. INTRODUCTION

Fungi have been a great spotlight as a biological control agent and large scale of work has been borne in order to find fungi with a good potential as a fungicides (Lopez, 1998). Some species of fungi have the abilities to secrete active substances that can be lethal to certain life form (Santamarina *et al*., 2002). Most fungal showed antagonistic effect to certain bacteria or fungi that cause plant disease. *Penicillium* spp. is one of the potential biological agent that capable to control certain pathogenic fungal species by inducing resistance in plant and by its metabolites (Narayanasamy, 2013). Besides, *Penicillium* spp. has been reported to have ability to suppress *Fusarium* and *Verticillium* wilt of tomato plant (Whipps, 2001). However, the use of fresh cells of potential biological control agent (BCA) have been widely studies in controlling plant disease (Mahdy *et al*., 1987; Larsen & Knechel, 1997; Cal & Melgarejo, 2000 and Shojaee *et al*., 2014). Even though it is effective and suitable for research purposes but it is not practical to be used by farmers. The inconsistency of the fresh cells of BCA makes it impossible for commercialization and longer time storage.

Formulated biological control products must be able to retain their similar productivity to the fresh cells (Janisiewicz & Jeffers, 1997). The major concern of the commercialize products are the stability of the shelf life of biological control in the market. Baker & Henis (1990) highlight that a commercialize product must be able to retain their stability for at least one year and must be stored at room temperature. Apart from that, the formulated product must be easy to handle and have stability over a range of -5 to 35 ºC (Junaid *et al*., 2013). Formulation of the biological control is the mixture of active ingredients such as conidia with the inert material such as surfactant in order to modify the physical characteristics to more desirable form (Junaid *et al*., 2013). Thus, the aim of this project is to assess the shelf-life of *P. oxalicum* T3.3 conidia in different ratio of carrier and storage temperature. The present study will increase knowledge on the development of commercialize biological control products for agriculture purpose.

II. MATERIAL AND METHOD

2.1 Microorganism

P. oxalicum T3.3 used in this study was previously isolated from dragon fruit plant by Suhaila (2014). *P. oxalicum* T3.3 was grown on Potato Dextrose Agar (PDA) plate for 7 days at 30ºC.

2.2 Fermentation media

Mixture of peat, vermiculite and soya bean (PVS) (1:1:1, wt/wt/wt) were used as basal medium. PVS (12 g) with 10 mL of distilled water was prepared in 250 mL flask. Then, the mixtures were sterilized by autoclaving in autoclave at 121ºC for 15 min. The mixture was allowed to be cooled until it reaches room temperature before inoculation process.

2.3 Preparation of spore suspension

One disc of *P. oxalicum* T3.3 grown on PDA from 7 days culture was inoculated into PVS media and incubated at 28 ºC for 8 days. Afterwards, spores suspension was harvested and adjusted to 10^9 CFU/mL and the spore's suspension was used for the preparation of the bioformulation.

2.4 Preparation of carrier materials

Inert carriers used for bioformulation were biochar, biocompost, peat moss and vermiculite. The carrier were divided into two mixture (biochar and compost; peat and vermiculite). Grounded carriers were further screened under 1 mm sieve to get fine powder form for inoculation. The fine powder were then oven- dried at 70 ºC for 48 hours and placed in an airtight polyethylene bags for sterilization in autoclaved at 121 ºC for 20 minutes in consecutive days before used for fungi inoculation.

2.5 Development of bioformulation at different storage temperature

Two combination of sterile powder bioformulation with the same ratio of carrier were prepared. Each mixture bioformulation; Peat:Vermiculite (PtVm) and Biochar:Biocompost (BcBp), 100 g each were mixed with 1 g of carbomethylcellulase (CMC) as additives. The mixture were inoculated under sterile condition with 40 mL of inoculum suspension after autoclaved at 121 ºC for 20 min (Vidhyasekaran & Muthamilan, 1995). The concentration of inoculum at initial was adjusted to 10^9 CFU/mL. Bioformulations were then stored at 4ºC and room temperature for 6 months.

2.6 Development of bioformulation at different ratio

Carriers were divided into two mixture; Peat:Vermiculite (PtVm) and Biochar:Biocompost (BcBp). Each carrier was mixed with different ratio at 1:1, 2:1 and 1:2 with the total weight of 100 g. The mixture were inoculated under sterile condition with 40 mL of inoculum suspension after autoclaved at 121 ºC for 20 minutes (Vidhyasekaran & Muthamilan, 1995). The concentration of inoculum at initial was adjusted to 10^9 CFU/mL. Bioformulations were then stored for 6 months.

2.7 Viability assessment of shelf life of bioformulation

The bioformulation were stored at different temperature at 4 ºC and room temperature and assessment were carried out for 6 months by monitoring the viability of the antagonistic fungi in the formulation. One gram of the sample was drawn from each formulation periodically at 0, 4, 8, 12, 16, 20 and 24 weeks of storage time and was mixed with 9 mL of sterile saline water. From this, serial dilution was made. One mL sterile saline water was added to three replicate tubes per treatment and spore concentration was adjusted about 1 x 10^7 spores/mL. Suspensions were plated onto three replicate petrifilm (1 mL per petrifilm) and were incubated at 30 ºC for 72 h. The numbers of germinated spores were counted in each petrifilm (3M) and colony forming unit was calculated. The viability lost was determined by comparing with the initial concentration, CFU/mL.

$$\text{CFU of sample} = \frac{\text{Average colonies x Dilution factor}}{\text{Sample size}}$$

III. RESULTS AND DISCUSSION

There were significant differences in survival of *P. oxalicum* T3.3 among the carriers and storage temperatures at initial day to 180 days. Formulation of BcBp and PtVm were able to retain the viable cells of log 5 and 4 CFU/mL for 180 days, respectively. At 4 ºC, viable cells of BcBp carrier was decreased significantly throughout the assessment. During 180 days of storage time, the viable cells were recorded with the value of log 5.43±0.13 CFU/mL (Figure 1). At room temperature, the numbers of viable cells in BcBp formulation sharply decreased at 30 to 60 days and then significantly decrease toward the end of assessment. The CFU unit recorded for BcBp at room temperature at the end of experiment was log 3.67±0.56 lower than at 4ºC. The results showed that BcBp was able to retained higher CFU at 4 ºC.

Fig. 2 shows that the viable cells in PtVm bioformulation were significantly declined throughout the experiment for both storage temperatures. At room temperature, viable cells were dropped from log 7.80±1.13 CFU/mL to log 5.50± 0.71 CFU/mL during 60 to 90 days storage time. Viable cell were decreased significantly from 120 to 180 days of storage with log 3 CFU/mL at the end of experiment. As for 4 ºC, the number of viable cell were constant from 90 to 120 days and start to decreased slowly until the end of assessment. The number of viable cells detected in 4 ºC was log 4.46±0.15 CFU/mL which was higher than at room temperature after 180 days. Statistically, this study suggested that there were significant differences in CFU count among the bioformulations at the end of the experiment with $P \leq 0.05$.

Fig. 3 and Fig. 4 show that there were no insignificant differences in viability cell at the end of experiment. BcBp at ratio 2:1 showed the highest cell viability with log 5.63±0.47 CFU/mL whereas 1:1 ratio at log 5.43±0.13 CFU/mL and followed closely by 1:2 with log 5.40±0.09 CFU/mL. PtVm also do not have a significant difference between ratios after 180 days. Ratio 2:1 gave the lowest viable cell with log 4.49±0.20 CFU/mL while 1:1 has the highest viable cell with log 4.79±0.71 CFU/mL and ratio 1:2 was recorded with log 4.69±0.36 CFU/mL. This study showed that different in ratio of carriers do not affect significantly on the viable cell at the end of assessment. Statistically, this study suggested that there were significant differences in CFU count among the bioformulations with $P \leq 0.05$.

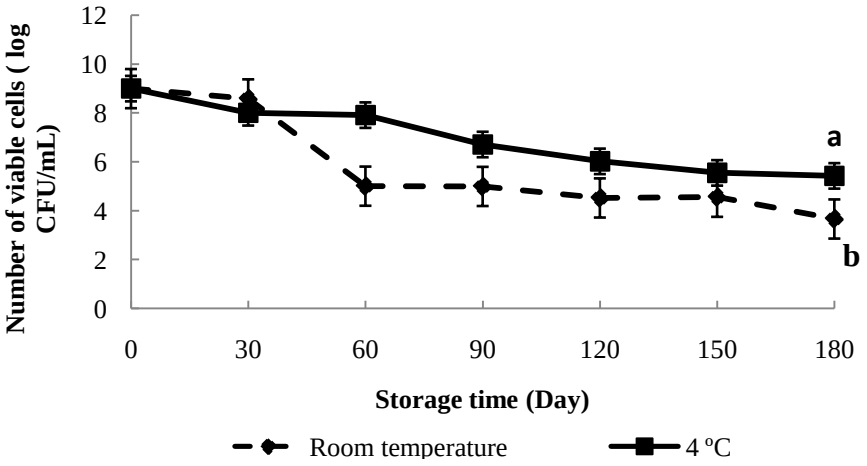

FIGURE 1: Survival of *P. Oxalicum* T3.3 in the Mixture of Biochar+Biocompost (BcBp) Bioformulation at Different Temperature.

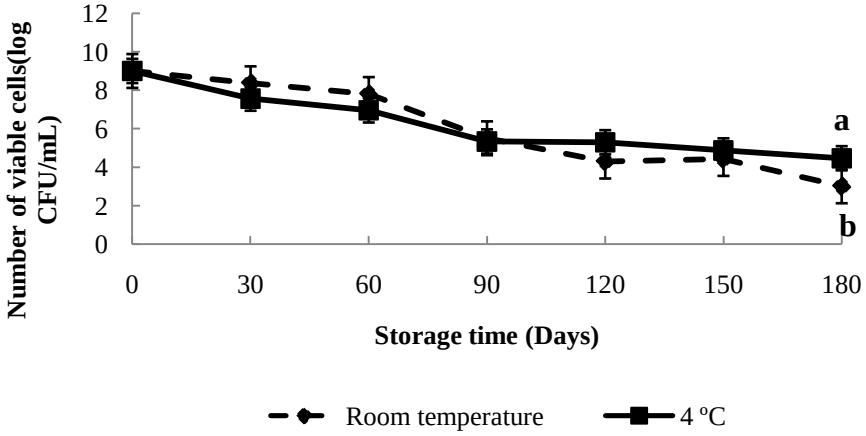

FIGURE 2: Survival of *P. Oxalicum* T3.3 in the Mixture of Peat+Vermiculite (PtVm) Bioformulation at Different Temperature.

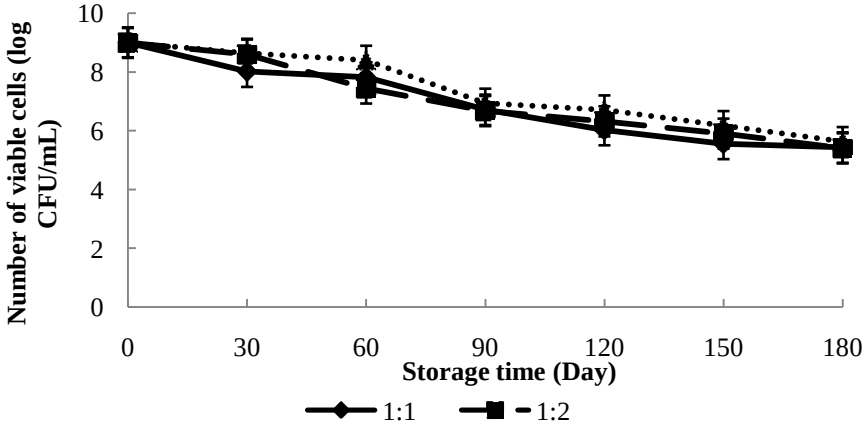

FIGURE 3: Survival of *P. Oxalicum* T3.3 in the Biochar+Biocompost (BcBp) Formulation at Different Ratio.

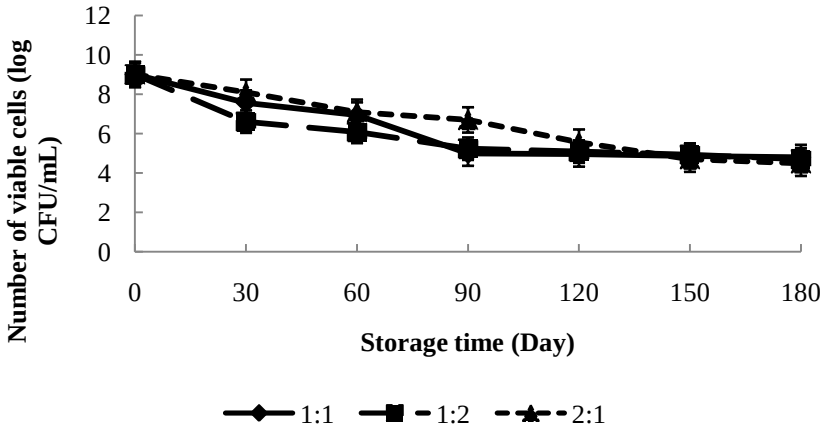

FIGURE 4: Survival of *P. Oxalicum* T3.3 in the Peat+Vermiculite (PtVm) Formulation at Different Ratio.

The initial viable cells in this study were higher than recorded by Pimenta *et al* (2008). *P. oxalicum* T3.3 populations were dropped at 30 days because of the lack of nutrient and moisture of the carriers. This is because when the fungi were transitioning from logarithmic to stationary phase, the P_{total}, N_{total}, K_{total} and the moisture content decreased due to the storage condition and microbial activities (Tate, 2000). During 120 days, death rate was slowly decreased until 180 days storage time. In this study, the survival of *P. oxalicum* T3.3 in PtVm and BcBp were less than those reported by other researches due to different types of carrier used (Bazilah *et al*., 2011; Singh *et al*., 2014).

The viability of cells in both formulation were higher at 4 ºC instead of room temperature because of the moisture availability surrounding the cells (Bazilah *et al*., 2011). Many of beneficial microbes have longer shelf-life when stored at lower temperature (Balume *et al*., 2015; Phiromtan *et al*., 2013). Low temperature also reduced water loss in the formulation and preserves the efficiency of fungi. Apart from that, high storage temperature help microbial growth which causes the microbial to produce more wastes (Bazilah *et al*., 2011). It also has been proved by Soe & De Costa (2012) where powder based formulation were able to maintain high cell viability over long time at 4 ºC and room temperature compared to spore suspension.

The results indicated that BcBp was the most suitable carrier for production *P. oxalicum* T3.3. The mixture of biochar and biocompost (BcBp) showed able to survive the viable cell of the inoculum after 180 days. Both of the carrier have microporous structure which is good to be used as a carrier (Somarathne *et al*., 2013). Biocompost and biochar itself have a beneficial nutrient which provided a good microbial habitat and help to prolong their survival rate. Apart from that, it promote the growth of plants by the interaction of plant microbial (Warnock *et al*., 2007). BcBp has high surface area because of their porous structures and it help to attract and absorb water. Thus, nutrients that good for microbial growth such as phosphorus and nitrogen also retained (Somarathne *et al*., 2013).

Peat and vermiculite (PtVm) were also considered as a good carrier since both of the carriers can be used as microbial inoculant alternative with each other. The mixture of both carriers help in improved microbial growth, promote seed germination when it used as seed treatments, and improved plant growth and yield. Nehra & Choudhary (2015) claimed that the used of peat as carrier was significantly reduces threat that resulting from contamination. Vermiculite was a good carrier because of the multilamellate structure that provided good aeration and space for microbial proliferation. Apart from that, it has good sticking properties which help to amend with peat as carrier and it also help the number of viable cells does not change significantly when stored at room temperature (Daza *et al*., 2000). BcBp performed well in preserving the *P. oxalicum* T3.3 population. Besides that, traditional carrier, PtVm also able retained a good number of viable cells even though it has lower number of viable cells than BcBp. Statistically; both formulations show a significant result at the end of assessment.

IV. CONCLUSION

In conclusion, both bioformulation were able to retained high CFU number during storage temperature 4 ºC compared to room temperature for 180 days. In comparison, BcBp bioformulation has higher cell viability with log 5.43±0.13 CFU/mL whereas PtVm bioformulation with log 4.46±0.15 CFU/mL. Bioformulation was able to preserve high cell population because of the presence of moisture surrounding the cells which help to reduce water loss in the formulation. Apart from that,

the both bioformulation also were tested for their effect on the different carrier ratio and study found that BcBp were able to maintain high CFU with log 5.63±0.47 CFU/mL at 2:1 while ratio 1:1 of PtVm recorded high CFU with 5.43±0.13 CFU/mL. A good carrier choice plays an important role in preserving cell viability. Present study showed that mixture of biochar and biocompost as good carriers for microbial habitat compared to peat and vermiculite.

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REFERENCES

[1] Baker, C. A., & Hennis, J. M. S. (1990). Commercial production and formulation of microbial biocontrol agents. In `New directions in biological control: Alternatives for suppressing agricultural pests and diseases'. R.R. Baker and P.E. Dunn. Alan R Liss, New York.

[2] Balume, I., Keya, O., Karanja, N., & Woomer, P. (2015). Shelf-life of legume inoculants in different carrier materials available in East Africa. Afr. Crop Sci J. 23(4), 379. http://doi.org/10.4314/acsj.v23i4.7

[3] Bazilah, A. B. I., Sariah, M., Abidin, M. A. Z., & Yasmeen, S. (2011). Effect of carrier and temperature on the viability of during storage. Int. J. of Agri. and Biol., 13, 198–202.

[4] Cal, A. De, & Melgarejo, P. (2000). Induced resistance by *Penicillium oxalicum* against *Fusarium oxysporum* f . sp . *lycopersici* : Histological studies of infected and induced tomato stems.Biol. Control, 90(3), 260-268.

[5] Daza, A., Santamaría, C., Rodríguez-Navarro, D. N., Camacho, M., Orive, R., & Temprano, F. (2000). Perlite as a carrier for bacterial inoculants. Soil Biol. and Biochem. 32(4), 567–572. http://doi.org/10.1016/S0038-0717(99)00185-6

[6] Janisiewicz, W. J., & Jeffers, S. N. (1997). Efficacy of commercial formulation of two biofungicides for control of blue mold and gray mold of apples in cold storage. Crop Prot. 16(7), 629–633. http://doi.org/10.1016/S0261-2194(97)00048-3

[7] Junaid, J. M., Dar, N. A., Bhat, T. A., Bhat, A. H., & Bhat, M. A. (2013). Commercial biocontrol agents and their mechanism of action in the management of plant pathogens. Int. J. of Mod. Plant & Anim. Sci. 1(12), 39–57.

[8] Larsen, a G., & Knechel, S. (1997). Antimicrobial activity of food-related *Penicillium* sp. against pathogenic bacteria in laboratory media and a cheese model system. J. of Appl. Microbiol. 83(1), 111–119. http://doi.org/10.1046/j.1365-2672.1997.00196.x

[9] Lopez, S. P. (1998). Studies on production and ecophysiology of fungal antagonists for improved biological control of *Fusarium oxysporum* f. sp. *Iycopersici* and *Monilinia laxa*. PhD Thesis. University Cranfield. Retrieved from http://dspace.lib.cranfield.ac.uk/handle/1826/4663

[10] Mahdy A. M., Sagitov, A. O. & Ahmed, G. A. (1987). Biological control of cucumber *Fusarium* wilt disease using *Chaetomium* and *Penicillium* isolates under protected houses. 87–92

[11] Narayanasamy, P. (2013). Biological management of diseases of crops. Springer Publisher. http://doi.org/10.1007/978-94-007-6380-7

[12] Nehra, V., & Choudhary, M. (2015). A review on plant growth promoting rhizobacteria acting as bioinoculants and their biological approach towards the production of sustainable agriculture. J. of Appl. and Nat. Sci. 7(1), 9411.

[13] Phiromtan, M., Mala, T., & Srinives, P. (2013). Effect of various carriers and storage temperatures on survival of *Azotobacter vinelandii* NDD-CK-1 in powder inoculant. Modern. Appl. Sci. 7(6), 81–89. http://doi.org/10.5539/mas.v7n6p81

[14] Pimenta, R. S., Silva, F. L., Silva, J. F. M., Morais, P. B., Braga, D. T., Rosa, C. A., & Jr, A. C. (2008). Biological control of P*enicillium italicum* , *P . digitatum* and *P . expansum* by the predacious yeast *Saccharomycopsis schoenii* on oranges, Brazillian J. of Microbiol. 39, 85–90.

[15] Santamarina, M. P., Roselló, J., Llacer, R., & Sanchis, V. (2002). Antagonistic activity of *Penicillium oxalicum* Corrie and Thom , *Penicillium decumbens* Thom and *Trichoderma harzianum Rifai* isolates against fungi , bacteria and insects in vitro, Rev. Iberoam. de Micol. 19, 99–103.

[16] Shojaee, N., Shahidi, B. G. H., Shahdaei, S., & Leyla, S. B. (2014). Biological control of citrus green mould, *Penicillium digitatum*, by antifungal activities of *Streptomyces* isolates from agricultural soils. Afr. J. of Microbiol. Res. 8(14), 1501–1509. http://doi.org/10.5897/AJMR2013.6289

[17] Singh, S., Gupta, G., Khare, E., Behal, K. K., & Arora, N. K. (2014). Effect of enrichment material on the shelf life and field efficiency of bioformulation of *Rhizobium* sp . and P-solubilizing *Pseudomonas fluorescens*, Sci. Res. Rep. 4(1), 44–50.

[18] Soe, K. T., & De Costa, D. M. (2012). Development of a spore-based formulation of microbial pesticides for control of rice sheath blight. Biocont. Sci. and Technol. 22(6), 633–657. http://doi.org/10.1080/09583157.2012.676025

[19] Somarathne, R., Yapa, P., Yapa, N., & Selection, A. S. (2013)." Use of different carrier materials for culture and storage of native forest soil microorganisms," unpublished.

[20] Suhaila, Mamat. (2014). Isolatioan and characterization of antifungal activity of endophytic *Penicillium oxalicum* T3. 3 for anthracnose biocontrol in dragon fruit (*Hylocereus* sp.). Master thesis, Universiti Putra Malaysia.

[21] Tate, R. L. (2000). Soil Microbiology. USA: John Wiley & Son, Inc.

[22] Vidhyasekaran, P., & Muthamilan, M. (1995). Development of formulations of *Pseudomonas fluorescens* for control of Chickpea wilt. Plant Dis. 79(8), 782-786. http://doi.org/10.1094/PD-79-0782

[23] Warnock, D. D., Lehmann, J., Kuyper, T. W., & Rillig, M. C. (2007). Mycorrhizal responses to biochar in soil - Concepts and mechanisms. Plant and Soil, 300(1–2), 9–20. http://doi.org/10.1007/s11104-007-9391-5

[24] Whipps, J. M. (2001). Microbial interactions and biocontrol in the rhizosphere. J. of Exp. Bot. 52(1), 487–511. http://doi.org/10.1093/jexbot/52.suppl_1.487.

Aflatoxin B1 Exposure Induced Obesity/Breast Cancer Based in Neighborhood Socioeconomic Status

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Running title: AFB1-induced Ob/BC in nSES.

Abstract—

Background: *Obesity (Ob) linked to Breast Cancer (BC) has reached epidemic proportions worldwide (WW) including Indonesia, Brazil, Bangladesh and also developed countries. To assess the prevalence and associated risk factors of central Ob and BC WW, are commonly linked with geography (wet and warm climate), race, immigrant, but not SES.*

Problem: *Low and middle-SES are associated with lower outcome affect late diagnosis, late hospitalization and treatment, but not with p53 mutation caused by AFB1 exposure.*

Objectives: *Low and middle-SES and neighborhood are linked to AFB1 exposure as the cause of Ob/BC prevalence.*

Method: *PRISMA design of Systematic Review using Science Direct and EBSCO data based with keywords Ob/BC AND SES. A Bayesian network of Ob (BMI, WC, WHR) and SES (neighborhood) are used. Mainly high AFB1 high guideline AFB1 exposures are recorded.*

Result: *One flowchart detected 152 references and one table or 30 references which included 153,099 subjects at least supported SES/AFB1-associated Ob/BC prevalence or almost related.*

Conclusion: *Low and middle-SES represent AFB1 exposure as the cause of Ob/BC beyond geography, race and ethnic.*

Keywords— Aflatoxin B1, Waist Circumference, Obesity, Breast cancer, socioeconomic status (SES), Neighborhood.

I. INTRODUCTION

Background/Aims: Prevalence Fat distribution due to Brown Adipose Tissue known as UCP2 abundant in Abdominal Adipose Tissue, UCP3 abundant in skeletal muscle, and UCP1 abundant in subscapular fat, is positively related with inflammation marker and the prevalence of Metabolic Syndrome (MS)-associated with pre diabetes, diabetes and its complication such as cardiovascular diseases.[1,2,3]Problem**:** Socioeconomic status (SES) are correlated with Ob-BC and Menopausal status (Estrogen),[4] neighborhood[5] and funding to early diagnostic,[6,7] treatment[8,9,10]and Physical Activity,[11,12]but Not AFB1 exposure are in the National government project. Socioeconomic change BC in Brazil in this 21 century.[13]Objective: AFB1 exposure marked by urine AFM1 sub ppb,[14,15,16,17] become the infrastructure of the cause and avoidance of SES problem positively related with Ob/BC high prevalence. AFB1 exposures are well known cause Liver Cancer, but almost never cause Ob/BC. Unlike that SES-associated better fund in early Ob/BC diagnosis, management, treatment project, a study SES-associated better management in AFB1 exposure in combatting Ob/BC. Abundance data of AFB1-Liver cancer will be useful for Ob/BC.

II. METHOD

Systematic Review design by PRISMA using Science Direct and EBSCO host data based and keyword Obesity/Breast Cancer (Ob/BC) AND socioeconomic status or SES. With a Bayesian network analysis Ob also marked by Waist Circumference (WC)/Waist Hip Ratio (WHR)/Metabolic Syndrome (MS)/diabetes mellitus (DM). Geography, race and minority are also use to be low and middle-SES which is broadly known as high AFB1 exposure (Brazil, Bangladesh, Hispanic, rural/urban etc).

III. RESULT

One flowchart to identified 152 references, which support Ob/BC AND SES/Aflatoxin exposure. Thirty References supported AFB1/SES-associated Ob/BC. Flowchart of the 30 identified Literatures on the association of SES and Ob/BC & SES and Aflatoxin are shown in Figure 1. Thirty References support Urine AFM1 sub ppb as a metabolite of AFB1 (SES) and Ob/BC high prevalence could be seen in Table 1. Not only in developed countries, Ob/BC and various risk factors are reported in Race/ethnic, SES and different geography area disparities.[18,19,20,21,22,23,24,25,26,27,28,29,30,31,32].

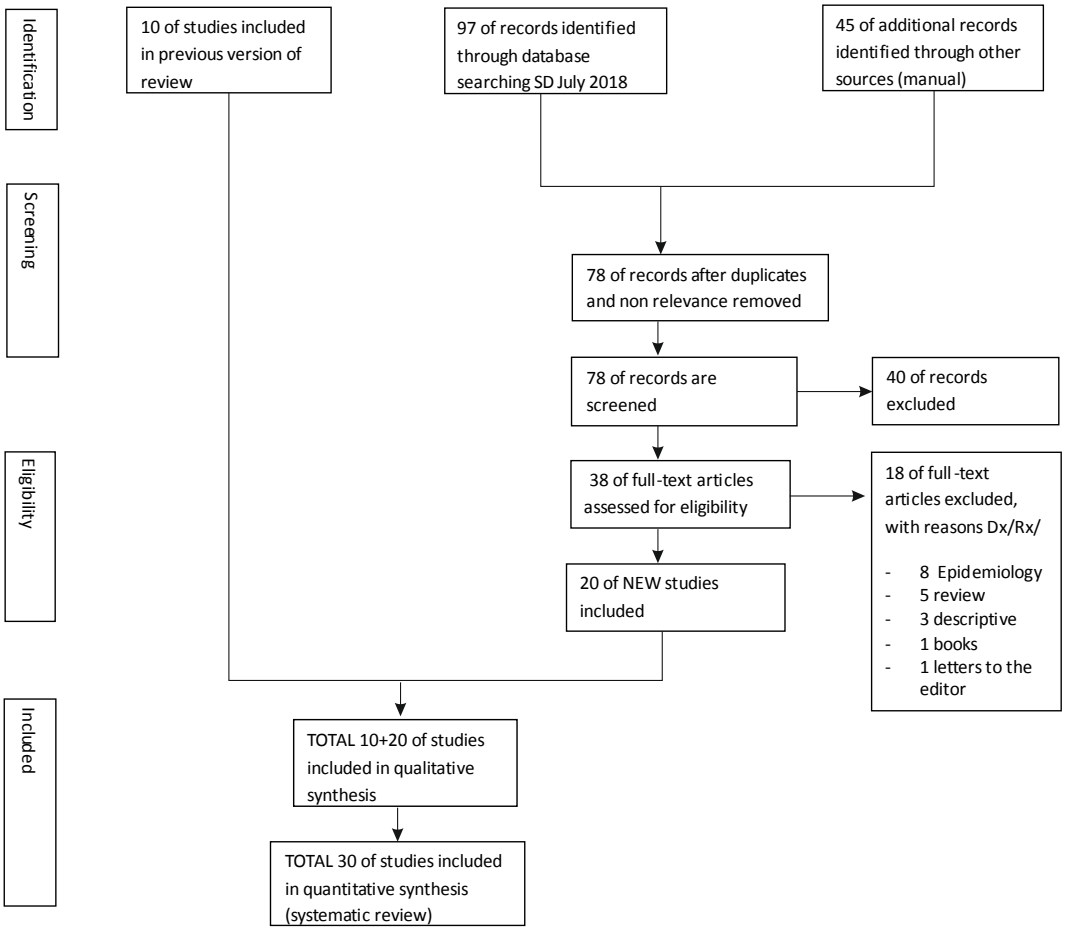

FIGURE 1. Flowchart of the 30 identified Literatures on the association of SES^Ob/BC and SES^Aflatoxin

TABLE 1
THIRTY REFERENCES SUPPORT URINE AFM1/ AFB1 (SES) AND OB-BC HIGH PREVALENCE

Study	Design	Population	Ob/BC	AFB1/*SES	Specific Definition
Shariff-Marko 2015	Descriptive	4347 SF Bay Area Dx/BC	WHR	Lower *SES, crowding	Social and built environment
Shariff-Marko 2017	Prospective Cohort	4,505 BC survivors N California	Ob/Ca	PA/diet	The Rich ##AA. US born Hispanic
Vigen 2016	Case control & survivor cohort	1,936 BC N California	BC	*SES or Education Multiethnic	DM, HT, MI, other heart diseases
Conroy 2017	Multiethnic cohort	48,247 postme-nopausal LA	Ob-BC risk	**nSES	Obesogenic
Conroy 2017	Case-control	1,838 cases 3,117control	BC risk	**nSES, Education	Race/Ethnic, urban, nativity
Padovani 2016	Descriptive Cross-sectional	40 Ob women	55% not aware of BC risk, None identify their own Ob	57,5 %made mammography annually	Unknown their Ob as a risk factor for BC
Espina 2017	Systematic Review	Low Africa High White	High mortality BC	Africa & Delays Dx/	Meta-analysis were not possible
Figueiredo 2018	Ecology study	Brazil 2004-2014	BC P Correlation, Linear Regression	Inequality income	Mortality, not the cause
Figueiredo 2018	Ecological study	Brazil 2004-2014	BC mortality increase Hospital adm rate increase	Socioeconomic development	Age-Hospi admission rates, mortality

Gomez 2015	Distribution	Northern California BC pts.	Lowest and highest SES	Academic medical center vs. integrated medical center	Suburban metropolitan area
Cheng 2015	Cross-sectional	B,995 BC California	BMI/Ob/BC	**nSES), Race/ethnic	PA, healthy eating
Figueiredo 2017	Secondary analysis	Brazil 2004-2014	BC	BC cost Regression	Health policy Bi implicative
Ali 2017	Descriptive & Cohort	218 Urine AFM1 rural-urban Bangladesh	AFB1 high risk exposure	HPLC-FD for Urine AFM1	Hot and Humid Climate: 1
Ali 2016	Descriptive	43 Urban-52 Rural Urine AFM1 Bangladesh	Food & Feed	Rural 99, Urban 54 pg/ML	Metabolite/ biomarker 31-348 pg/mL
Ali 2016	Descriptive	24 AFs spices in market Malaysia	Spices common ingredients in Asian	AFs 8,38 ng/g AFB1 7,31ng/g (>5 ng/g)	HPLC-FD LOD 0,01 ng/g
Gerding 2015	Descriptive	Bangladesh Germany Haiti	References; Ob/BC prevalence and risk	Urine AFM1 only in Bangladesh and Haiti	LC-MS/MS Tandem Individual Detection
Alam 2016	Exploratory study	1243 Undiagnosed/vs. Neverdiagnosed/ Bangladesh	Similar to WHR	Low-income settings	T2D and pre-diabetes
Sposto 2016	Cohort	12.098 BC	54% BC ##NHW	Lifestyle	Race/ethnic disparity
Wu 2015	Multiethnic Cohort	8,952 BC California	Body size/DM mortality	Race/ethnic similarity	Ca-spec mortality
Wee 2016	Cross-sectional	963 LSES 1060 control Singapore	Cardiovascular Ca screening	Low *SES rental flat	Breast Ca Ca Cervix
Wee 2000	Population-based Survey	11,435 Singapore	OvW/Ob/Mortality rate BC	Less screen	Social & Psychological
Sabran 2012	Descriptive	160 Urine AFM1 Malaysia	Milk & Dairy > median 67,79 g/day: significant high level of urine AFM1	Ref. permissive AFs Malaysia (>5ng/g)	61,3% n 0,0234 ng/mL urine AFM1
Sabran 2016	RCT Probiotic Cross-over	71 Employee Univ Malaysia	Ref.: Ob/BC risk	Reduce AFB1 serum & urine AFM1	Yellow & Blue group longer warranty
Sabran 2013	A Mini review	Peanuts, cereal, spices Malaysia	AFs greater than the permissible limit, postulated moderate	AFs exposure recorded in Malaysia since 1960,	Foodstuffs and human AFs detected
Rachmi 2016	Cross-sectional	4101 2-4,9 y Indonesian, 13 of 27 prov.	Overweight or Obesity BMI	Risk factors Low and middle-income	Z-score uW, stunted Doubleburden
Mitchell 2016	Cohort & Observation	Nepal age 15, 24, 36 month	Childhood stunting	AFB1-lysine	g-mean 3,62 pg/mg alb
Moss 2017	Surveillance, Epidemiology, End Result	#NHW 2009-2013 UR	Cancer incidence Urban BC >& CC<	Ecological analysis SES	Weighted at least Sq-Reg
Owusu 2018	Cohort	60 Older ##AA, NHW (66-87y)	Survivor BC	*SES	Physical Activity> Race
Rutherford 2015	Cohort	England Wales BC pts.	Relative survival	*SES	Life expectancy
Kweon 2017	Surveillance, Epid, End R	10,528 pts Korea incl. BC	Ca Dx/incl. BC	*SES	Stage at Ca Dx/

SES: Socioeconomic Status; **nSES: neighborhood SES;#NHW: Non Hispanic White;##AA: African America

IV. DISCUSSION

AFB1/ inflammation induce UCP2/Ob-associated BC and MS/Pre-MS is associated with BC. Education and SES is recorded to be AFB1 exposure. Geography, Race, rural/urban should not be the infrastructure fighting undiagnosed DM, pre-DM, Ob-associated BC. Obesity-BC were associated with neighborhood SES.[4,5]Not only WHRas a marker of Ob-BC associated with low income,[18] but also middle income-associated BMI,WC, Cardiovascular risk[34] also a marker of Ob/BC. Breast Cancer cases in integrated health care[10]also associated with SES[10]and survival BC[12]but not the prevalence BC is related to SES which is associated with recreational Physical Activity.

4.1 AFB1 exposure induce Ob-BC

AFB1 is inflammation stuff induce UCP2 function as antioxidant present central Ob which associated with BC. Central Ob, UCP2, BMI that represent Adipose Tissue distribution positively related with CRP and other inflammation marker.[35,36,37]Abdominal fat (represented by WC) as antioxidant of inflammation in Diabetes and Pre Diabetes,[38] is in high prevalence in rural Bangladesh[39] was widely known as central Obese. Central Ob is associated also with Metabolic Syndrome (MS) and pre MS in health professional in Brazil,[35] also in wet and warm climate area such in rural Bangladesh. Urine AFM1 as a metabolite of AFB1 exposure are also in high prevalence in Brazil,[20,40,41,42,43] and Bangladesh.[17,15]

4.2 Specific of General Knowledge bring to controversies which still not clear

Latin America and China export maize all over the world. There are paired of nations (developing-developing, developed-developed countries) trading large amounts of maize. Paired countries have very similar aflatoxin regulations: nations with strict standards tend to trade maize with each other, while nations with more relaxed standards tend to trade maize with each other. Rarely among the top pairs of maize-trading nations do total aflatoxin standards (standards based on the sum of the levels of aflatoxins B1 etc.), more than 5 μg/kg.[44] So, it is reported that globally, countries with the same standard level has separate maize trading communities. These nations tend to trade with other nations that have very similar food safety standards. These evidence based report failed to proof that Developed countries still have Ob-BC are due to AFB1 exposure came from developing countries maize export merchandise shipment network.[44] Socioeconomic status positively correlated with Ob/BC but related to late diagnostic and late therapy not with AFB1 exposure.[13] Metabolic Syndrome and pre MS in health professional means middle SES also have AFB1 exposure, and urine AFM1 support that the middle socioeconomic income people still not aware of AFB1 exposure. So do Hispanic vs. non Hispanic White women which still significant higher prevalence in Ob, BMI and WC which is associated with ER negative, not associated AFB1 exposure.[45] In Latin America, also where AFB1 could be associated with BC high prevalence.[46] Aflatoxin regulation in a Network of Global Maize Trade has fail to proven the AFB1 exposure in low- and middle-socioeconomic class in developed countries.[20] Epidemiology is inconsistent to prove Ob/BC, but WC are associated with BC and menstrual status,[47,48] which Estrogen and fat distribution control AFB1 exposure.[49] The conclusion of the lost of weight not the avoiding of AFB1 exposure once again need the urine AFM1 sub ppb to convince people the AFB1 exposure.[11,48]

4.3 AFB1-induced Ob/BC

While Ob is well-understood to increase BC risk, high prevalence in AFB1 exposure which induce central Ob and TNBC in wet and warm area or low and middle socioeconomic status are proven. Geography, race/ethnic are positively related with neighborhood, such as in Hispanic American,[45] Latin America,[46] and population who has large WC in association with high BC prevalence masked by Menstrual status,[47,48] low and middle SES neighborhood bring us to the risk of Ob/BC expose by AFB1. Central Ob marked by WC is in high prevalence in rural Bangladesh.[39] are parallel with urine AFM1 high prevalence in Brazil.[41,42,43] Further the role of AFB1-Neighborhood SES/crowded that influence body size and adipose tissue distribution should account for obesogenic environment,[4] independent of racial/ethnic.[5] Targeted prevention effort of BC could be use that potentially be averted if all women attained a BMI less than overweight and obese.[50]

V. LIMITATION

Study design Meta-analysis could not be trapped to build this Systematic Review. Simple characterized low and middle-SES as AFB1 exposure are bridged by WC and/ or Ob/BC need furtherAFB1 exposure intervention. We need prevention of AFB1 exposure through urine AFM1 sub ppb marker to convince people without awareness in their not healthy food.[14,15,41,42,43] Confuse definition of exposure AFB1 and other mycotoxin and different outcome variable such as survival, surveillance, mortality rate but not the prevalence of BC, spread the focus of AFB1 exposure as the cause of Ob/BC in fighting it.[33-40]

VI. CONCLUSION

Not only in wet and warm known as developing countries, but also low and middle SES population in developed (dry and cold) countries, the food and feeds have AFB1 exposure high risk, and this inflammation stuff is the cause of Ob/BC has been masked by estrogen in premenstrual age.

CONFLICT OF INTEREST

No Conflict of Interest till the date.

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REFERENCES

[1] Shariff-Marco S, Gomez SL, Sangaramoorthy M, Yang J, Koo J, Hertz A, et al. Impact of neighborhoods and body size on survival after breast cancer diagnosis. Health Place 2015;36:162–72.

[2] Sharif-Marco S, Von Behren J, Reynolds P, Keegan THM, Hertz A, Kwan ML, et al. Impact of Social and Built Environment Factors on Body Size among Breast Cancer Survivors: The Pathways Study. Cancer Epidemiol Biomarkers Prev A Publ Am Assoc Cancer Res Cosponsored By Am Soc Prev Oncol 2017;26(4):505–15.

[3] Vigen C, Kwan M, John E, Gomez S, Keegan T, Lu Y, et al. Validation of self-reported comorbidity status of breast cancer patients with medical records: the California Breast Cancer Survivorship Consortium (CBCSC). Cancer Causes Control 2016;27(3):391–401.

[4] Conroy SM, Clarke CA, Yang J, Shariff-Marco S, Shvetsov YB, Park S-Y, et al. Contextual Impact of Neighborhood Obesogenic Factors on Postmenopausal Breast Cancer: The Multiethnic Cohort. Cancer Epidemiol Biomarkers Prev A Publ Am Assoc Cancer Res Cosponsored By Am Soc Prev Oncol 2017;26(4):480–9.

[5] Conroy SM, Shariff-Marco S, Koo J, Yang J, Keegan THM, Sangaramoorthy M, et al. Racial/Ethnic Differences in the Impact of Neighborhood Social and Built Environment on Breast Cancer Risk: The Neighborhoods and Breast Cancer Study. Cancer Epidemiol Biomarkers Prev A Publ Am Assoc Cancer Res Cosponsored By Am Soc Prev Oncol 2017;26(4):541–52.

[6] Padovani C, Tomeleri da Fonseca Pinto KR, dos Santos Laqui I, Ferrari Bernardy CC, Carreira CM. RISK FACTORS FOR BREAST CANCER: KNOWLEDGE BY A GROUP OF OBESE WOMEN. J Nurs UFPE / Rev Enferm UFPE 2016;10(7):2319–27.

[7] Espina C, McKenzie F, Dos-Santos-Silva I. Delayed presentation and diagnosis of breast cancer in African women: a systematic review. Ann Epidemiol 2017;27(10):659–671.e7.

[8] Figueiredo FWDS, Almeida TC do C, Cardial DT, Maciel É da S, Fonseca FLA, Adami F. The role of health policy in the burden of breast cancer in Brazil. BMC Womens Health 2017;17(1):121.

[9] dos Santos Figueiredo FW, Adami F. Income Inequality and Mortality Owing to Breast Cancer: Evidence From Brazil. Clin Breast Cancer 2018;18(4):e651–8.

[10] Gomez SL, Shariff-Marco S, Von Behren J, Kwan ML, Kroenke CH, Keegan THM, et al. Representativeness of breast cancer cases in an integrated health care delivery system. BMC Cancer 2015;15:688.

[11] Cheng I, Shariff-Marco S, Koo J, Monroe KR, Yang J, John EM, et al. Contribution of the neighborhood environment and obesity to breast cancer survival: the California Breast Cancer Survivorship Consortium. Cancer Epidemiol Biomarkers Prev A Publ Am Assoc Cancer Res Cosponsored By Am Soc Prev Oncol 2015;24(8):1282–90.

[12] Keegan THM, Shariff-Marco S, Sangaramoorthy M, Koo J, Hertz A, Schupp CW, et al. Neighborhood influences on recreational physical activity and survival after breast cancer. Cancer Causes Control CCC 2014;25(10):1295–308.

[13] dos Santos Figueiredo FW, Cardial DT, do Carmo Almeida TC, da Silva Cardial C, de Carvalho LEW, Adami F. Socioeconomic changes in Brazil impacted breast cancer indexes at the beginning of the 21st century? J Cancer Policy 2018;16:39–42.

[14] Ali N, Hossain K, Blaszkewicz M, Rahman M, Mohanto NC, Alim A, et al. Occurrence of aflatoxin M1 in urines from rural and urban adult cohorts in Bangladesh. Arch Toxicol 2016;90(7):1749–55.

[15] Ali N, Blaszkewicz M, Hossain K, Degen GH. Determination of aflatoxin M1 in urine samples indicates frequent dietary exposure to aflatoxin B1 in the Bangladeshi population. Int J Hyg Environ Health 2017;220(2 Pt A):271–81.

[16] Ali N, Hashim NH, Shuib NS. Natural occurrence of aflatoxins and ochratoxin A in processed spices marketed in Malaysia. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2015;32(4):518–32.

[17] Gerding J, Ali N, Schwartzbord J, Cramer B, Brown DL, Degen GH, et al. A comparative study of the human urinary mycotoxin excretion patterns in Bangladesh, Germany, and Haiti using a rapid and sensitive LC-MS/MS approach. Mycotoxin Res 2015;31(3):127–36.

[18] Alam DS, Talukder SH, Chowdhury MAH, Siddiquee AT, Ahmed S, Pervin S, et al. Overweight and abdominal obesity as determinants of undiagnosed diabetes and pre-diabetes in Bangladesh. BMC Obes 2016;3:19.

[19] Sposto R, Keegan THM, Vigen C, Kwan ML, Bernstein L, John EM, et al. The Effect of Patient and Contextual Characteristics on Racial/Ethnic Disparity in Breast Cancer Mortality. Cancer Epidemiol Biomarkers Prev A Publ Am Assoc Cancer Res Cosponsored By Am Soc Prev Oncol 2016;25(7):1064–72.

[20] Wu AH, Kurian AW, Kwan ML, John EM, Lu Y, Keegan THM, et al. Diabetes and other comorbidities in breast cancer survival by race/ethnicity: the California Breast Cancer Survivorship Consortium (CBCSC). Cancer Epidemiol Biomarkers Prev A Publ Am Assoc Cancer Res Cosponsored By Am Soc Prev Oncol 2015;24(2):361–8.

[21] Wee LE, Cher WQ, Sin D, Li ZC, Choon-Huat Koh G. Primary care characteristics and their association with health screening in a low-socioeconomic status public rental-flat population in Singapore- a mixed methods study. BMC Fam Pract 2016;17:1–14.

[22] Wee CC, McCarthy EP, Davis RB, Phillips RS. Screening for cervical and breast cancer: is obesity an unrecognized barrier to preventive care? Ann Intern Med 2000;132(9):697–704.

[23] Tangka FK, Subramanian S, Mobley LR, Hoover S, Wang J, Hall IJ, et al. Racial and ethnic disparities among state Medicaid programs for breast cancer screening. Prev Med (Baltim) 2017;102:59–64.

[24] Mohd-Redzwan S, Jamaluddin R, Abd-Mutalib MS, Ahmad Z. A mini review on aflatoxin exposure in Malaysia: past, present and future. Front Microbiol 2013;4:334.

[25] Mohd Redzwan S, Abd Mutalib MS, Wang J-S, Ahmad Z, Kang M-S, Abdul Rahman N 'Aqilah, et al. Effect of supplementation of fermented milk drink containing probiotic Lactobacillus casei Shirota on the concentrations of aflatoxin biomarkers among employees of Universiti Putra Malaysia: a randomised, double-blind, cross-over, placebo-controlled ... Br J Nutr 2016 ;115(1):39–54.

[26] Mohd Redzwan S, Rosita J, Mohd Sokhini AM, Nurul Aqilah AR. Association between aflatoxin M1 excreted in human urine samples with the consumption of milk and dairy products. Bull Environ Contam Toxicol 2012;89(6):1115–9.

[27] Rachmi CN, Agho KE, Li M, Baur LA. Stunting, Underweight and Overweight in Children Aged 2.0-4.9 Years in Indonesia: Prevalence Trends and Associated Risk Factors. PLoS One 2016;11(5):e0154756–e0154756.

[28] Mitchell NJ, Riley RT, Egner PA, Groopman JD, Wu F. Chronic aflatoxin exposure in children living in Bhaktapur, Nepal: Extension of the MAL-ED study. J Expo Sci Environ Epidemiol 2016;

[29] Wyatt TE, Pernenkil V, Akinyemiju TF. Trends in breast and colorectal cancer screening among U.S. adults by race, healthcare coverage, and SES before, during, and after the great recession. Prev Med Reports 2017;7:239–45.

[30] Moss JL, Liu B, Feuer EJ. Urban/Rural Differences in Breast and Cervical Cancer Incidence: The Mediating Roles of Socioeconomic Status and Provider Density. Women's Heal Issues Off Publ Jacobs Inst Women's Heal 2017;27(6):683–91.

[31] Owusu C, Antognoli E, Nock N, Hergenroeder P, Austin K, Bennet E, et al. Perspective of older African-American and Non-Hispanic white breast cancer survivors from diverse socioeconomic backgrounds toward physical activity: A qualitative study. J Geriatr Oncol 2018;9(3):235–42.

[32] Rutherford MJ, Andersson TM-L, Møller H, Lambert PC. Understanding the impact of socioeconomic differences in breast cancer survival in England and Wales: avoidable deaths and potential gain in expectation of life. Cancer Epidemiol 2015;39(1):118–25.

[33] Kweon S-S, Kim M-G, Kang M-R, Shin M-H, Choi J-S. Difference of stage at cancer diagnosis by socioeconomic status for four target cancers of the National Cancer Screening Program in Korea: Results from the Gwangju and Jeonnam cancer registries. J Epidemiol 2017;27(7):299–304.

[34] Patel SA, Ali MK, Alam D, Yan LL, Levitt NS, Bernabe-Ortiz A, et al. Obesity and its Relation With Diabetes and Hypertension: A Cross-Sectional Study Across 4 Geographical Regions. Glob Heart 2016;11(1):71–79.e4.

[35] Vidigal F de C, Ribeiro AQ, Babio N, Salas-Salvadó J, Bressan J. Prevalence of metabolic syndrome and pre-metabolic syndrome in health professionals: LATINMETS Brazil study. Diabetol Metab Syndr 2015;7:6.

[36] Samsuria P, Abdullah M, Wahid M, Hartati M, Darminto M, Sabarguna6 B, Nurani S. UCP2's fat cells proliferation in insulin resistance high prevalence in unfortunate developed and developing countries populations: p53 mutation-increase IGF-1. Proceeding 5th WC CODHy, Istanbul-Turkey 2015; 63. URL

[37] Sam S, Haffner S, Davidson MH, D'Agostino Sr RB, Feinstein S, Kondos G, et al. Relation of abdominal fat depots to systemic markers of inflammation in type 2 diabetes. Diabetes Care 2009;32(5):932–7.

[38] Siddiquee T, Bhowmik B, Da Vale Moreira NC, Mujumder A, Mahtab H, Khan AKA, et al. Prevalence of obesity in a rural Asian Indian (Bangladeshi) population and its determinants. BMC Public Health 2015;15(1):860.

[39] Siddiquee T, Bhowmik B, Karmaker RK, Chowdhury A, Mahtab H, Azad Khan AK, et al. Association of general and central obesity with diabetes and prediabetes in rural Bangladeshi population. Diabetes Metab Syndr 2015;9(4):247–51.

[40] Saldan NC, Almeida RTR, Avíncola A, Porto C, Galuch MB, Magon TFS, et al. Development of an analytical method for identification of Aspergillus flavus based on chemical markers using HPLC-MS. Food Chem 2018;241:113–21.

[41] Cássia A De, Raquel T, Ferreira B, Tadeu C, Calori-domingues MA, Micotti E. Occurrence of AFM 1 in urine samples of a Brazilian population and association with food consumption. J FOOD Control 2009;6–10.

[42] Jager A V, Tonin FG, Souto PCMC, Privatti RT, Oliveira CAF. Determination of urinary biomarkers for assessment of short-term human exposure to aflatoxins in São Paulo, Brazil. Toxins (Basel) 2014;6(7):1996–2007.

[43] Jager A V, Tonin FG, Baptista GZ, Souto PCMC, Oliveira CAF. Assessment of aflatoxin exposure using serum and urinary biomarkers in São Paulo, Brazil: A pilot study. Int J Hyg Environ Heal 2016;219(3):294–300.

[44] Wu F, Guclu H. Aflatoxin Regulations in a Network of Global Maize Trade. PLoS One. 2012;7:10–3.

[45] Connor AE, Baumgartner RN, Yang D, Slattery ML, Giuliano AR, Risendal BC, et al. Differences between Hispanic and non-Hispanic white women with breast cancer for clinical characteristics and their correlates. Ann Epidemiol 2013;23(4):227–32.

[46] Amadou A, Torres-Mejía G, Hainaut P, Romieu I. Breast cancer in Latin America: global burden, patterns, and risk factors. Salud Publica Mex 2014;56(5):547–54.

[47] His M, Dartois L, Fagherazzi G, Boutten A, Dupré T, Mesrine S, et al. Associations between serum lipids and breast cancer incidence and survival in the E3N prospective cohort study. Cancer Causes Control CCC 2017;28(1):77–88.

[48] Pichard C, Plu-Bureau G, Neves-E Castro M, Gompel A. Insulin resistance, obesity and breast cancer risk. Maturitas 2008;60(1):19–30.

[49] Gérard C, Brown KA. Obesity and breast cancer - Role of estrogens and the molecular underpinnings of aromatase regulation in breast adipose tissue. Mol Cell Endocrinol 2018;466:15–30.

[50] Engmann NJ, Golmakani MK, Miglioretti DL, Sprague BL, Kerlikowske K. Population-attributable risk proportion of clinical risk factors for breast cancer. JAMA Oncol. 2017;3(9).

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