RESEARCH PROPOSAL (Extension for possible funding)

I. BASIC INFORMATION

Program/Project/Study Title: STANDARDIZATION, REFORMULATION, AND UTILIZATION OF ORGANIC FERTILIZERS IN VSU (*ORP04 - EFS 3.1418-1*)

Program/Project/Study Leader(s): Romel B. Armecin, Suzette B. Lina

Implementing Unit: Ecological Farm and Resource Management Institute (Eco-FARMI), Visayas State University

Cooperating/ Collaborating Agency(ies): None

Location: Eco-FARMI Demonstration Farm

Duration: January 2022 to December 2024

Proposed Budget: Php645,000 @ Php 215,000 per year

Discipline: Soil Science

Classification: Basic/Applied

II. TECHNICAL INFORMATION

A. Rationale

In the past, the use of synthetic agri-chemicals has raised a number of ecological problems. In recent years, however, scientists have diverted their attention towards exploring the potential of beneficial microbes for plant protection measures and those organisms that enhances nutrient availability. Bio-control is a strategy that is easy to deliver, improve plant growth, activate resistance mechanisms in the host, and increase biomass production and yield. These antagonists act through antibiosis, secretion of volatile toxic metabolites, mycolytic enzymes, parasitism and through competition for space and nutrients. Likewise, application of beneficial organisms enhances nutrient uptake through the substance and enzymes produced in a form of mucilage (e.g. glomalin in mycorrhizal fungus) and by

increasing the root volume of the plant brought about by the hyphal network formed by the fungus.

In modern agriculture, fertilizers are necessary materials because they provide essential plant nutrients. However, overuse of fertilizers can cause unanticipated environmental impacts. The search for microorganisms that improve soil fertility and enhance nutrient availability has continued to attract attention due to increasing cost of fertilizers and some of their negative environmental impact. One potential way to decrease negative environmental impacts resulting from continued use of chemical fertilizers is through the inoculation of plant growth promoting microorganisms (PGPM's). These organisms exert beneficial effects on plant growth and development, and many different endophytes have been commercialized for use in agriculture. One of the important mechanisms for these beneficial effects is PGPM-elicited enhanced nutrient availability and nutrient use efficiency. These types of bio-organic fertilizers are considered as effective and one of the approaches used in wide array of organic agriculture production systems.

In the advent of the organic agriculture program of the country, various organic fertilizer products are produced with varying level of formulations. Most of these products ranged from the liquid to granulated forms, and composted products such as vermicast. These are the commonly used and readily available organic fertilizers in the local market. However, the composition of these products in terms of nutrient contents and microbial population present are unknown to the end-users specifically the farmers. Supposedly, this information (e.g. labels in each product) should be the basis in drawing up recommendation to the existing condition of the field to be grown with a particular crop and the amount to be applied based on the nutrient contents of the product. The absence of the data on the contents of the organic fertilizer would lead to an erroneous and hit-and-miss recommendation by the field technician in-charge. Hence, this proposal would try to evaluate and assess the composition of the locally produced organic fertilizers and to enhance its composition by inoculating with PGPM. Likewise, the most promising fertilizer material will be assessed for better growth and yield performance of field grown crops.

B. Objectives

The overall objective of this project is to produce an effective organic fertilizer products in VSU that has the potential for commercialization

Specifically this project aims to:

a. Standardize and improve the quality of organic fertilizer produced at the EcoFARMI vermicomposting facility with the use of locally available wastes;
b. Assess the growth and yield performance of organically grown crops through the application of improved organic fertilizer products;
c. Develop IEC materials and disseminate information related to the reformulated organic fertilizer products.

C. Logic Framework (See Attached sheet)

D. Review of Literature

Commercial application of organic fertilizers either to increase crop health or to manage plant diseases depend on the development of commercial formulations with suitable carriers that support the survival of micro-organisms for a considerable length of time. The organic carriers used for formulation development include peat, turf, talc, lignite, kaolinite, pyrophyllite, zeolite, montmorillonite, alginate, press mud, sawdust, and vermiculite. Carriers increase the survival rate of the organism by protecting it from desiccation and death of cells (Heijnen et al., 1993).

Increase in public concern about the environment has increased the need to develop and implement effective organic fertilizer to enhance crop uptake. Likewise, an effective organic fertilizer could be developed for disease control only after understanding its performance in the environment in which it is expected to perform. In nature, agricultural crops are exposed to diverse environmental conditions which alter the microclimatic that would cause disease problems. A thorough knowledge on the mechanisms and performance related

to disease control will help in the selection of promising candidates that suits industries to produce reliable commercial products (Collins et al., 2003).

Organic fertilizers for the control of soil-borne pathogens

Introduction of organic fertilizer strains to phyllosphere, spermosphere or rhizosphere may be moderately effective or sometimes totally ineffective under field conditions to control plant diseases (Duffy et al., 1996). Inefficacy of the strains under field conditions may be due to the variation in climatic conditions that suppress growth and survival of biocontrol agents (Guetsky et al., 2001). In addition both pathogen and bio-control agents does not have similar ecological niche for their growth and survival. Hence the efficacy of bio-control agents could be improved through the usage of compatible mixed inoculum of bio-control agents which could have a consistent performance under diverse environmental conditions (Guetsky et al., 2001; Janisiewicz, 1996).

Organic fertilizer formulations comprising of beneficial organisms such as fungus and bacterial strain mixtures having the capability to induce chitinase in plant play an important role in hydrolyzing chitin. These are structural components in gut linings of insects and would lead to better control of insect pest (Broadway et al., 1998). In addition, certain PGPM strains also activate octadecanoid, shikimate and terpenoid pathways. This in turn alters the volatile production in the host plant leading to the attraction of natural enemies (Bell and Muller, 1993). Identification of entomopathogenic organic fertilizer strains that have the capability to colonize phylloplane in a stable manner will be a breakthrough in the management of foliar pesticides (Otsu et al., 2004). Combined application of entomopathogenic strains with compatible PGPM strains that have the ability to suppress plant diseases has to be developed for broad spectrum action.

Amidst these obstacles, since organic fertilizer has its own potentiality in plant disease and pest management, several products have been registered for the practical use of farming community. Sixty to 75% of cotton crops raised in U.S. are treated with commercial product of B. subtilis(Kodiak) effective against soilborne pathogens such as Fusarium and Rhizoctonia. It is also used in peanut, soybean, corn, vegetables and small grain crops (Backman et al.,

1997). In China, PGPM has been in commercial development for more than two decades and are referred as yield increasing bacteria (YIB). It is applied over an area of 20 million hectares of different crop plants (Chen et al., 1996; Kilian et al., 2000). In India, more than 40 stakeholders from different provinces have registered for mass production of organic fertilizer (Ramakrishnan et al., 2001). Though the market size for organic fertilizer usage is increasing constantly under greenhouse and field conditions, finding solutions for the above obstacles will create a breakthrough in the adoption of bio-control agents for field applications.

Beneficial organisms as nutrient availability enhancers

Organic fertilizer, an alternative source of chemical fertilizer, can substantially supplement the N requirement and enhance the uptake of water and mineral nutrients by the host plants. Recently, organic fertilizers are gaining prominence because they play an important role in the maintenance of soil fertility. Rhizobacteria are able to promote growth and yield of agriculturally important crops grown under different soil and climatic conditions (Okon and Labandera-Gonzales, 1994). Crops rely mainly on the N2 fixation process by associative, symbiotic and free living bacteria in the rhizosphere (Cocking, 2000). Graminaceous plants potentially are capable of establishing associations with diazotrophic bacteria where Azospirillum provide the host with a source of N (Brimecombe et al., 2001). Plant growth promoting rhizobacteria(PGPR) inoculation can provide 31% N requirement in maize and 40% of the N requirement for oil palm seedlings through biological nitrogen fixation (BNF) under glasshouse condition (Amir et al.,2001; El-Komy et al., 1998). In field condition, rice can obtain 20% of its N and sugar cane 70% of its total N requirement through N2 fixation (Boddey et al., 1995; Shrestha and Ladha, 1996).

In banana, Wange and Patil (1994) found an increased plant growth by PGPM inoculation together with N-fertilizer but could not show root development and yield improvement. Application of PGPM can save up to 67% of the total requirement of N in sweet potato (Saad et al., 1999) and 48% in oil palm seedlings (Amir et al., 2001). In general, N2 fixation is increased with modest levels of soil or fertilizer-N, but declines at high N levels

because of mineral-N depress N2 fixation (Marschner, 1995). A lower but specific level of fertilizer-N might be required for optimum N2 fixation by PGPM in association with banana roots. Although there are many beneficial effects of PGPM to the host plant, their survival and effectiveness as N2 fixer and bio-enhancer depend upon various factors such as successful colonization by the microorganism, synergistic effect of inoculation with fertilizer N and availability of other plant nutrients (Mia et al., 2010).

E. Expected Output

Improved the quality of fertilizer material and better growth of horticultural crops

F. Potential Impact:

Increase farmer's income, improved growth of horticultural crops and encourage farmers to use BOF

G. Methodology/ Work plan

Vermiculture study with the use of locally available substrates

Collection of raw materials

Substrates such as cow manure, kakawate, rice straw will be used in this experiment. Carabao manure will obtained from Eco-FARMI vermicomposting facility, while kakawate and rice straw will be collected in the nearby surrounding. This will be brought into a screenhouse facility and will be allowed to sun-dry for twenty-one (21) days. This will be done in order to remove the moisture in each substrate. After sun-drying, kakawate and rice straw will be shredded at Eco- FARMI vermicomposting facility. Prior to mixing, three (3) subsamples from each substrate will be collected as representative samples for chemical analysis.

Similarly, samples of additional substrates such as jackfruit and vegetable wastes will be collected from the nearby area. Representative samples will be collected for moisture content determination and basis for the determination of different ratios added in the original/recommended mixtures. Chemical analysis will also be done just like in the previous substrates mentioned earlier.

Establishment and Experiment Set-up

This will be conducted at the Eco-FARMI vermicomposting facility in order to know the effects of Jackfruit and vegetable wastes on the worm population and quality of vermicast. The experiment will be laid out in a 2x2x3 factor factorial experiment arranged in Randomized Complete Block Design (RCBD) with three (3) replicates.

The different treatments are designated as follows:

Factor A (Recommended substrates)

S_1 = Cow manure: Kakawate: Rice straw

S_2 = Mudpress: Kakawate: Rice straw

Factor B (Type of substrate added)

A_1 = vegetable wastes

A_2 = jackfruit

Factor C (Ratio of the added substrate)

R_1 = 0%

R_2 = 100%

R3 = 200%

The experiment will be conducted using styrobox with a dimension (55cm x 35cm x 9cm L X W X H) fitted with a black net. The recommended substrates will be mixed at a ratio of 4:2:1 by 500g dry weight basis. Thus, the additional substrates (i.e. vegetable wastes and jackfruit) will be added as percentages (0, 100 and 200%, respectively) based on the dry weight basis from the recommended/original substrates. After mixing the original substrates, this will be watered 2x a week at rate of 2L of water and will be allowed to pre-decompose for twenty one (21) days. After the pre-decomposition period, fresh additional substrates will be added to each designated treatments. Then 200 worms will be added in each experimental unit. The set-up will be watered two (2) times a week at a rate of 1 L water or when necessary. The experiment will be terminated after two (2) months of composting period:

In this study, the following parameters will be gathered:

a. Moisture and dry matter content of the substrates. The moisture content of the different substrates will be determined by taking 100g of each substrate samples in the aluminum box and placed in the oven for 48 hours. The samples will be kept at 105°C until it attained a constant weight. Then, the sample will be cooled, first in the switched-off oven. The

cooled sample will be weighed. The loss in weight will be equal to the moisture contained in 100-g substrate sample. The percentage of moisture will be calculated as:

$$Percent\ Moisture\ (\%) = \frac{Fresh\ weight - Ovendry\ weight}{Fresh\ weight}\ x\ 100$$

The oven dry weight of each substrate will also determined following the same procedure above for the computation of the rate of decomposition. The oven dry weight was computed as:

$$Dry\ matter\ content\ (\%) = 100 - moisture\ content$$

b. Rate of degradation/decomposition. The rate of degradation/decomposition of the different substrates will be determined by computing the oven-dry weight of the combined degraded material over the initial dry weight of the same combined substrates after 50 days of observation. The harvested cast will be passed through a 4mm mesh-sieve to separate the decomposed from the undecomposed materials. The rate of degradation will be computed in percentage using the formula:

$$Rate\ of\ Degradation/Decomposition\ = \frac{ODW\ of\ DS}{ODW of\ US}\ x\ 100$$

where:

ODW = Oven Dry Weight (g)
US = Undecomposed substrates
DS = Decomposed Substrates

c. Vermicast Recovery. This will be obtained by sieving the collected vermicast derived from the combination of the different substrates and will be weighed to determine the amount of vermicast produced. Vermicast recovery will be computed in percentage using the formula:

$$Vermicompost\ Recovery\ (\%) = \frac{Harvested\ Vermicast\ (kg)}{Total\ weigth\ of\ substrate\ (kg)}\ x\ 100$$

d. Weight and number of earthworms. The weights (initial and final) and number of earthworms will be recorded from day 0 and 50 days of decomposition.

At the termination, three (3) vermicasts subsamples will be taken for the analysis of the following parameters

a. Moisture content (%) = This will be determined using the following the formula:

$$\% MC = (FW-ODW)/ODW \times 100$$

where:
MC = moisture content (%)
FW = weight of fresh organic fertilizer (g)
ODW = weight of oven-dried organic fertilizer (g)

b. pH (Potentiometric method) - This will be determined using potentiometric method (PCARR,1980). A 10g air-dried sample that passed through a 2mm sieve or number 10 mesh screen will be placed into a 50ml beaker. The vermicast sample will be added with 10 ml distilled water, stirred thoroughly and will be allowed to stand for 15 min after mixing. Immediately before placing the electrode of calibrated pH meter, then suspension will be stirred thoroughly.

c. EC (electrical conductivity) - This will be analyzed potentiometrically using a soil-water ratio of 1:5 (ISRIC 1995). A 10-g air-dried sample which passed through a 2-mm mesh sieve will be weighed in a plastic cup, then it will be added with 50-mL distilled water. The solution will be stirred thoroughly until a suspension will be formed. It will be allowed to stand for one hour and will be stirred before reading in a pre-calibrated electrical conductivity meter

d. Organic Matter (%)- This will be determined following the Walkley-black method (Nelson and Sommers, 1982). A 0.5g sample that has passed through 0.425 mm sieve (screen# 40) will be transferred into a 500ml erlenmeyer flask. Using a volumetric pipet, a 10 ml 1 N will be added and the flask will be swirled gently to disperse the sample in the solution. Under the hood, a 10ml concentrated sulphuric acid will be added rapidly, directing the stream into the suspension. The flask will be swirled immediately to stand under the hood for 1 hour. Then 200 ml distilled water will be added. Four drops of O-phenanthroline indicator will be added, the solution will be stirred and titrated with 0.5 N as the endpoint

approach, the solution will change from greenish cast to dark green. Ferrous sulphate heptahydrate will be added drop by drop until the color changes sharply from blue to red. The organic matter will be computed using the formula:

$$\%OM=10\ (1\ S/B)\ \times 0.69\ X\ 1/W$$

Where:

OM= organic matter
S= ml of $FeSO_4.7H_2O$ in soil sample titration
B= ml of $FeSO_4.7H_20$ in blank titration
W= weight of soil sample (g)

d. Total Nitrogen (%) - This will be analyzed using the Micro-Kjeldahl method (ISRIC, 1995). A 0.5 g of sample that passed through a 0.425 mm mesh will be added with 0.5 g selenium mixture. Then, 5 ml of concentrated H_2SO_4 will be added and the samples will be digested using Kjeldahl digestion heaters. Distillation will be done using Buchi distiller apparatus wherein 25 ml 40% NaOH will be added. Fifty (50) ml of the distillate will be collected in Erlenmeyer flask containing 10 ml H_3BO_3 (2%) and 2-3 drops of mixed indicator. The distillate will then be titrated using the standardized 0.05 N H_2SO_4 until the color changes from green to pink. Percent (%) N will be calculated as follows:

$$\%N=((a-b))/S \times 0.05\ N\ H_2SO_4 \times 1.4 \times mcf$$

Where:
a = volume (mL) of standardized H_2SO_4 for titration of sample,
b = volume (mL) of standardized H_2SO_4 for titration of blank,
s = weight (g) of the samples,
N = normality of standardized H_2SO_4
mcf = moisture correction factor

e. Total Phosphorous (%) - This will be determined using Aqua Regia method (Chen and Lena, 2001) wherein 0.1 g sample that has passed 2-mm wire mesh will be mixed and will be digested with 10 ml concentrated HCl and 5 ml of concentrated nitric acid placed in a microwave digester then the sample will be diluted with deionized water and volume to 50 ml.

The extract will be quantified following Murphy and Riley method (19852). A 2 ml aliquot of the extract from each treatment will be placed in test tubes and was added with mixed reagent and stand for one hour to develop the molybdenum blue color. The sample will be measured using B-L spectronic 20 at 880 nm and computed using the formula:

Total P (%)=ODS x K x 100/0.05 x 1/10,000 x dilution

Where:
ODS= optical density
K= slope of standard curve
100= dilution of digested sample
1/1000= to express result in % basis

g. Total Potassium (%) - The extract from aqua regia will be analysed for total K using AAS.

The experiment will be establish again using the same protocol mentioned above and using the same worm in order to observe the effects of Jackfruit after four (4) months.

Microbial Activity of Vermicast

One hundred grams of fresh sample will be placed in a glass. Fifteen mL of freshly prepared 1N NaOH in the 50 mL plastic centrifuge will be carefully placed in the glass jar. The glass jars will be tightly closed and sealed with tape and incubated at room temperature (approximately 27°C). The 50 mL plastic with alkaline trap will be removed from each glass jar after 24 hours, and every 7 days' interval. One (1) mL 0.5 N $BaCl_2$ in a 50 mL plastic centrifuge tube with NaOH will be added before and carefully transferred to an Erlenmeyer flask. Thereafter, 3-4 drops of phenolphthalein will be added to the solution and titrated until the endpoint is reached.

The amount of CO_2 evolved will be calculated using the following formula and the results will be expressed as mg CO_2 produced per 100 g sample.

$$Milligrams\ C\ or\ CO2 = (B - V)\ NE$$

where:

V = volume (mL) of acid to titrate the alkali in the CO_2 collectors from treatments to the endpoint
B = volume (mL) of acid to titrate the alkali in the CO_2 collectors from controls to the endpoint
N = Normality of the acid

E = equivalent weight. If data are expressed in terms of carbon (E = 6, if expressed as CO_2, E = 22

Data analysis

Collected data will be encoded in Microsoft excel worksheet and will be converted to csv comma delimited file and will be statistically analyzed by using MSTATC software version 1.0. If found significant, treatment means will be compared using Duncan's Multiple Range Test (DMRT) at 5 % level of significance.

References:

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H. Milestone

Objectives	Expected Output	Activities	Months						
			1st	2nd	3rd	4th	5th	6th	7th

I. Users/ Target Beneficiaries:

J. Budget Requirement (Philippine Peso) Use separate template provided

Operational Definition of Terms:

Title – The identification of the program/ project and the projects/ component/ studies (as defined by DOST)

Program – consists of interrelated or complimenting R&D Projects on a multi-disciplinary approach to meet established goals within a specific time frame (as defined by DOST)

Project - a set of interrelated studies to meet pre-determined objectives within a specific time frame (as defined by DOST)

Study -

Leader – the one in-charge to take the lead in program/ project/ study implementation (as defined by DOST)

Researcher – refers to person working in those capacities, who uses or creates scientific knowledge and engineering and technological principles (as defined by DOST)

Cooperating/ Collaborating Agency(ies) – agencies participating in the R&D work

Duration – number of months the program/project/study will be implemented. To include date of implementation and completion

Classification – indicates whether the program/ project is a Research (basic, applied), Development or Extension program/ project/ study (as defined by DOST)

Basic Research – an experimental or theoretical work undertaken primarily to acquire new knowledge of the underlying foundations of phenomena and observable facts, without any particular or specific application or use in view(as defined by DOST)

Applied Research – is an original investigation undertaken in order to acquire new knowledge directed primarily towards a specific aim or objective (as defined by DOST)

Development – is a systematic work, drawing on existing knowledge gained from research and/or practical experience that is directed to producing new materials, products or devices, installing new processes, systems and services and improving substantially those already produced or installed

Discipline – the specific field to be studied

Users/ Target Beneficiaries – refers to the clientele

Personal Services (PS) – total requirement for wages, salaries, honoraria, additional hire and other personnel benefits (as defined by DOST)

Maintenance and Other Operating Expenses (MOOE) – total requirement for supplies, materials, travel expenses, communication and other services (as defined by DA-BAR)

LOGICAL FRAMEWORK

Project Title: STANDARDIZATION, REFORMULATION, AND UTILIZATION OF ORGANIC FERTILIZERS IN VSU (*ORP04 - EFS 3.1418-1*)

Narrative Summary	Project Targets – Objectively Verifiable Indicators	Means of Verification	Assumptions
Goal: To be able to produce an effective organic fertilizer products in VSU that has the potential for commercialization	(a) Improved and effective organic fertilizer products in VSU; (b) increased income of the farmers practicing organic agriculture	Living status and indices, Annual reports of line agencies	Market prices will remain favourable and stable; Farmers are willing to adopt the organic farming technologies
Purpose: (a) Standardize and improve the quality of organic fertilizer produced at the EcoFARMI vermicomposting facility with the use of locally available wastes; (b) Assess the growth and yield performance of organically grown crops through the application of improved organic fertilizer products; (c) Develop IEC materials and disseminate information related to the reformulated organic fertilizer products	Production of improved and effective organic fertilizer products will increase in the vermicomposting facility	Laboratory analysis result, photodoc, reports, and organic fertilizer production statistics	Continuing support of the government in the promotion of the organic agriculture program for sustainable crop production in the countryside.

Project Outputs: 1. Analyzed collected samples 2. Standardized and reformulated organic fertilizer products 3. Tested green leafy vegetables of the reformulated organic fertilizer products 4. Evaluated the effectiveness of reformulated organic fertilizer products 5. Published article in peer-reviewed journal	(a) 100% of the collected samples were analyzed (b) Documented and published brochures on organic fertilizer production (c) Increased farm productivity by 10% through the use effective organic fertilizer in target areas (d) Three articles published in peer-reviewed journal	Photodocs, Progress reports, published articles	Stakeholders are willing to participate in the survey, sampling, and evaluation in the target areas
Activities: 1. Analysis of organic fertilizer products at EcoFARMI Vermicomposting facility 2. Standardization and reformulation of organic fertilizer products 3. Field Evaluation on the effectiveness of organic fertilizer products 4. Report writing	Inputs: Supplies – 50,000 Maintenance – 50,000 Maintenancce – 150,000 Supplies – 50,000 Supplies – 150,000 Maintenance – 100,000 Supplies – 50,000	Vouchers, acknowledgement receipts, laboratory analysis results, data of the field trial experiments, brochures on organic fertilizer production, photodoc, and progress reports	a) On-time release of allocations in line with the proposed activities and workplans; b) Gov't remains committed to the various stakeholders involved for the promotion of the organic agriculture program; c) Farmers are willing to adopt the organic production technologies