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The Role of PHILIPPINE HEIRLOOMS in Sustainable FOOD SECURITY

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EDITOR'S NOTE

The Central Bicol State University of Agriculture (CBSUA) known as Camarines Sur State Agricultural College started its operation as a farm school since 1918. Over the years, this learning institution provided the rudiments of farming to high school students which evolved into an agricultural state college mandated to provide higher and advanced education in agriculture hand-in-hand with the generation, dissemination, and commercialization of agricultural technologies. With its Vision to be an Agricultural Research University of Global Standards, technologies have been developed through research efforts of its faculty and students focusing on Bicol heirlooms such as pili, chili, mushroom, taro, *Hibiscus*, and stingless bees, with the latter as the flagship program of the university. Thus, the theme of this maiden issue of the AGRIKULTURA: CBSUA Research and Innovation Journal (ACRIJ) is the "Role of Philippine Heirlooms in Sustainable Food Security".

For the past 100 years, the university survived as a community known to the Bicolanos as AGRIKULTURA being the only agricultural school/college converted into a university. Sustainable production of both plants and animals made the college popular. Even in locating the university- which is in Barangay San Jose Municipality of Pili, using the word "AGRIKULTURA" would easily and conveniently bring you to this community. However, because of its conversion into a university, the place "AGRIKULTURA" is slowly being replaced with CBSUA. Through this publication, we are reviving the word "AGRIKULTURA" being a place of bounty harvest and providing food security to its community and the nearby communities through its research and innovation initiatives. This journal is blessed with internationally renowned experts as Associate Editors in the fields of Agriculture and Fisheries, Socio-economics, Policy and Ethics, Agricultural Technology and Biosystems, Food Technology and Nutrition, Environmental Sciences, Genetics and Biotechnology, and Innovative Extension Modalities.

Together with the Editorial Board and the university officials and constituents, we are happy to share to the international landscape the technologies generated by the university. Succeeding issues will present articles from other researchers from other national and global universities, sharing technologies for sustainable food security.

Let me take this opportunity to thank the Associate Editors for accepting our invitation. Likewise, we are indebted to the peer reviewers of this volume namely: Dr. Ramon A. Razal, Dr. Shaikh Tanveer Hossain, Dr. Senaratne L. Ranamukhaarachchi, Dr. Manuel C. Palada, Dr. Ratcha Chaichana, and Dr. Ravindra C. Joshi, for their generous comments for the improvement of the articles. We also are grateful to the CBSUA Board of Regents led by CHED Commissioner Dr. Aldrin A. Darilag and the CBSUA University President Dr. Alberto N. Naperi for supporting us in this endeavor. Sincere thanks to Dr. Ravindra C. Joshi (Consultant), Dr. Ramona Isabel S. Ramirez (VP for Research and Innovation, Managing Editor), Julie Amara J. Mostoles, Alvir E. Bausa, and Mark Jaypee Gonzales for their valuable inputs. We thank the authors of the articles in this first issue for answering our call to publish their works.

MARIA DULCE J. MOSTOLES, Ph.D.
Editor-in-Chief

FROM THE DESK OF THE UNIVERSITY PRESIDENT

As part of the institutionalized research culture in the university that promotes discovery, development and delivery of knowledge and processes, the launching of the AGRIKULTURA-CBSUA Research and Innovation Journal comes at a very important time.

There is clearly an upsurge of renewed interest in agriculture brought about by the present global health and economic challenges. Particularly, the relevance of a research university on what it can provide and generate for its communities now and in years to come is demanded.

This inaugural issue which includes a number of seminar papers, serves as a forum for exchanging interdisciplinary scientific knowledge that is aimed to widen the academic stronghold and to strengthen the community engagements and productivity.

With this journal and its ensuing publications, it is not only its own place in the research arena that CBSUA tries to emphasize but to carry through its mission of leading innovations, building resilient and sustainable communities.

For this, I would like to thank the many people who created the opportunity for the journal to be launched: the CBSUA Board of Regents led by CHED Commissioner Dr. Aldrin A. Darilag, Dr. Maria Dulce J. Mostoles (Editor-in-chief), Dr. Ramona Isabel S. Ramirez (Managing Editor), the contributors, the internationally renowned Associate Editors and the CBSUA Editorial Support Staff.

May this cutting-edge endeavor continue to enrich lives for better tomorrows!

ALBERTO N. NAPERI, DPA
SUC President IV

UTILIZATION AND COMMERCIAL PRODUCTION OF STINGLESS BEES AND ITS PRODUCTS IN BICOL, PHILIPPINES

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Abstract — Upscaling meliponiculture can increase income through the utilization and commercial production of the bee and its products. This includes improved and sustained production with the abundant colony and pollen sources, enhanced pollination, and developing health and wellness products from stingless bees. The research objectives were: map out the geographical distribution, abundance, and morphological characterization of stingless bees in the Bicol Region; determine the pollen sources of bees in meliponaries and bloom pattern in the area; determine the pollination efficiency by stingless bees on pigeon pea; and, develop food and cosmetic products from stingless bees.

Stingless bee colonies were sighted in all provinces in Bicol. Geographical locations were: Albay (Daraga), Camarines Norte (San Lorenzo Ruiz), Camarines Sur (Goa, Iriga, Tinambac), Catanduanes (Viga, Bagamanoc, Panganiban), Masbate (Aroroy), Sorsogon (Bulusan, Casiguran, Pilar, Prieto Diaz) but most abundant in Albay. Open nested colonies with clustered brood are T. *sapiens* while closed nested colonies with spherical brood are T. *biroi.* Acetolyzed bee bread from different meliponaries confirmed the pollen sources and documented the plant species, raw pollen, acetolyzed pollen, and bloom pattern. Pollination efficiency with 15 adult bees on pigeon pea flowers was 96.88%. Food and cosmetic products using pollen, propolis, and honey that were developed include moisturizing creams, propolis sprays, hand sanitizers, bath soaps and shampoo bars, macaroons with honey, polvoron with pollen, and honey-propolis candies. Three (3) IEC materials were distributed in trainings, exhibits, and fora.

Keywords — **Stingless bees distribution, product utilization, pollen sources, pollination.**

INTRODUCTION

The Bicol region is blessed with two major indigenous species of bees, namely *Apis cerana* and *Tetragonula biroi* which are found abundantly in its forests. Numerous research have been done by the Central Bicol State University of Agriculture being the Regional Apiculture Center (RAC) in upscaling the beekeeping technology particularly the stingless bees which are indigenous in the region. Results of investigations paved the way to the development of technology on stingless bees, referred to as Meliponiculture. In 2013, cultural practices have been published by Mostoles.

The stingless bees locally known as "lukot" produce honey, propolis, pollen, Royal jelly, and wax. Numerous benefits derived from its products such as honey, propolis, and pollen have been documented. To wit: honey as an effective cure for asthma and other respiratory diseases, bee glue or propolis with antimicrobial properties, and bee pollen as a protein source. These products were likewise developed by the university such as the cream and astringent (Mostoles and Ruiz, 2010), but massive commercialization is still wanting. Investigations are being made on Royal jelly and wax. With the stingless bees' high survival and low absconding rates, the nucleus can be produced commercially, utilized for pollination contracts to generate additional income for food security, especially by the marginal farmers.

For the sustainability of this emerging agro-industry, utilization and commercialization efforts must be done. Technology adaptation and verification can be initiated by knowing the distribution of the stingless bees in the wild, its pollen sources, and the bloom pattern in meliponaries, crop pollination efficiency. When good apicultural practices are developed and the yield of by-products has increased, there will be a need to process and utilize these products either for food, medicine, or skincare. For the past 12 years, commercial production of *T. biroi* mushroomed in the region, with no less than 20 meliponaries established (Mostoles et al, 2015). However, only a few sustained the industry due to abiotic and biotic constraints. The availability of the feral colonies in some areas has been done and the species were identified. Morphological analysis of stingless bees collected from the provinces of Camarines Sur and Albay was carried out in 2008 with eight species noted and now with ongoing studies on the bioinformatics of stingless bees. The performance of stingless bees in different hives and ecosystems were evaluated. Pollen sources identified were coconut, guava, citrus, mango, pili, avocado, neem tree, star apple, jackfruit and banana, *Wedelia*, *Chromalaena*, *Mimosa*, gumamela, shanghai beauty, santan, eggplant, pepper, squash, corn, and other weed species. Previous documentation by Mostoles and Ruiz (2010) was done on pollination of *Jatropha,* effects of propolis on skin irritations, wounds, and scratches, but required clinical analysis.

No comprehensive study and documentation on the distribution, pollen sources, and pollination efficiency of the stingless bees is available in the region. This information could serve as a basis and useful guide for future beekeepers not only in the Bicol region but in other parts of the country. The objectives of the research were: a. Mapping the distribution and abundance of stingless bee in the wild and identify the species found in the different provinces of the region; b. Identify the pollen sources of stingless bees in the wild and in meliponaries; c. Determine the pollination efficiency of stingless bees on pigeon pea; and, d. Develop and improve food and cosmetic products from stingless bees.

MATERIALS AND METHODS

Distribution and Relative Abundance of Stingless Bees in the Bicol Region

All the provinces in the region were surveyed for the presence of feral colonies of stingless bees in the wild and meliponaries. Documentation of the nesting sites, hive entrance, and hive architecture was done, and 25 adult bees were collected for morphological examination and identification. Location (coordinates and elevation) of the nests were determined using the Garmin GPS 12XL with maps generated.

Pollen Sources of Stingless Bees and Bloom Pattern

Pollen from the bee bread was collected from meliponaries in Sorsogon (Castilla, Casiguran, PIlar), Masbate (Aroroy and San Pascual, Burias) , Catanduanes (San Andres), Camarines Norte (San Lorenzo Ruiz, Camarines Sur (Pamplona, Gainza, Bula, Pili), and Albay (Guinobatan, Ligao City). The strength of these apiaries differed based on the number of colonies cultured and the suitability of their surroundings. Acetolysis of the bee bread samples was done in the laboratory. A drop of glycerine was added to the acetolyzed sample, mounted on a slide, and examined under the microscope. Photo documentation of the identified raw and acetolyzed pollen was done. Flowering plants (trees, shrubs, etc) in the meliponaries were noted and the time of flowering was recorded.

Pollination of Pigeon Pea

Field evaluation of the pollination efficiency of pigeon pea by stingless bees was done at San Jose, Pili, Camarines Sur. There were five treatments: Treatment 1- Open type of pollination, the flower of pigeon pea is exposed to the pollinators such as wasp, butterfly, and bees; Treatment 2- No bees were introduced and the flowers were caged to represent the control; Treatment 3- Five (5) individuals of stingless bees were introduced per caged branch of pigeon pea bearing flower; Treatment 4- Ten (10) individuals of stingless bees were introduced per caged branch of pigeon pea bearing flower; Treatment 5 - Fifteen (15) individuals of stingless bees were introduced per caged branch of pigeon pea bearing flower. Before caging and introduction of bees, each branch serving as the sample was first evaluated by counting the number of closed flowers. For each treatment, the corresponding numbers of stingless bees were introduced inside the cage. For T1, the branches were labelled and the no. of flowers counted. For T2, the no. of flowers in the sample branch was counted and enclosed in the cage without any pollinators inside. Daily monitoring of the cage was done until the flowers were pollinated and until the fruit set was initiated. The pollinated flowers were counted as well as the total number of flowers and the percentage pollination efficiency was computed and documented.

Food and Cosmetic Products from Stingless Bees

Food products were developed making use of either or a combination of the by-products such as propolis, pollen, and honey. Sensory evaluation of the products followed by packaging and labelling were done. The project focused on developing cosmetic products such as soap, shampoo bar, hand sanitizers, and throat spray. Previously developed moisturizing cream was enhanced. As the research is on technology adaptation and verification, IEC materials were developed for distribution to potential adopters.

RESULTS AND DISCUSSION

Distribution and Relative Abundance of Stingless Bees in the Bicol Region

A distribution map of the stingless bees in the different Bicol provinces was generated through GIS Mapping and is presented in Figure 1. Nesting sites and brood types are presented in Figure 2. The description of the colonies is presented below.

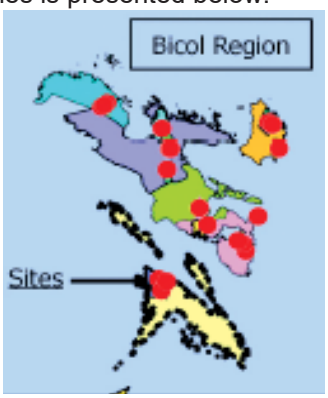

Fig. 1. Distribution of Stingless Bees in different provinces in the Bicol region.

A. Nest on coconut.

B. Brood types: L-spherical; R-cluster.

Fig. 2. Nesting sites (A) and Brood types (B) of Stingless Bees.

In the province of Albay, eight colonies were found in Barangay de la Paz (Daraga) with 1 nesting on hollow coconut (closed type, 123.6909°E, 13.13536°N) while 7 colonies on narra (open and closed nest,123.6957°E, 13.13413°N) At Brgy. Dinoronan, an open nested colony (123.6913°E, 13.13622°N) was found nesting on dried lanipga *(Toona kalantas)* tree. The mean distance between colonies is 0.396 km, considered as clump due to their closeness.

In the municipality of Aroroy (Masbate), Brgy. Matalang-talang had two clustered colonies in an old house (closed, 123.3486°E, 12.50322°N), Brgy. Cabangcalan, had two clustered colonies found in the kitchen (closed, 123.3692°E, 13.40157°N) and Brgy Pinanaan had two stingless bee colonies, one with spherical and the other clustered brood in the wall made of hollow blocks with protruding hive entrance (closed and open, 123.4143° E, 13.48182°N), two colonies (open and closed nesting). The mean distance of colonies was 9.912 km indicating a dispersed condition.

At Brgy. San Roque, Bulusan (Sorsogon), two open-nested spherical colonies were collected on coconut and betel nut (124.0819°E, 12.7541°N) while at in Brgy Del Rosario, Pilar, closed nesting spherical colonies were found in coconut. At Brgy. Tigbao (124.01549°E, 12.84297°N), and Brgy. Inlagadian (124.05908°E, 12.81576° N), both in Casiguran, closed nesting types with spherical brood nested on coconut were collected. At Brgy. Quidolog, Prieto Diaz (124.18708°E, 12.05975°N, and 124.05908°E, 12.81576°N), closed nesting and spherical brood colonies were found in the hollow sampaloc log. The mean distance of colonies from each other was 25.993 km indicating that it is widely dispersed.

At Brgy. San Pedro town of Goa (Camarines Sur), three colonies were

noted- all are spherical brood, in *Ficus nota* tree (open, 123.44736°E, 13.66064°N), in gumihan (*Artocarpus sericarpus*) tree (closed, 123.44149°E, 13.66923°N), and in lanipga tree (closed, 123.4345°E, 13.66089°N) Mean distance between colonies is 8.665km.

At barangay Matacong (Camarines Norte), a closed nesting and spherical brood colony hived in coconut (122.86342°E, 14.03032°N). The same type of colony was collected in Brgy. Mampurong (122.887306°E, 14.04695° N). At Barangay Dagotdotan, open nested spherical brood hived on narra (122.9116°E, 14.0676°N). The mean distance between colonies was 4.473 between colonies.

It was observed at Brgy. Ananong (Viga, Catanduanes) (124.30764°E, 13.87383°N), that a closed nesting colony hiving on a fern tree was observed to have an elongated hive. At Brgy. San Miguel (Panganiban) (124.27756°E, 13.89388°N), a closed nesting colony nesting in a hollow trunk of anislag tree observed to have an elongated hive entrance and clustered brood type. The same type of colony nesting in hollow narra trunk was seen at Brgy. Quezon (Bagamanok) (124.22912°E, 13.9371°N). A total of 6 colonies were sighted in four (4) municipalities with a mean distance of 5.719 between each nest of stingless bees.

Identity of the Stingless Bees

Morphological characteristics of the collected colonies of stingless bees were measured which served as basis in the identification of the species. The closed nesting colonies and spherical brood types were identified to belong to the genus *Tetragonula , carbonaria* group while the open nesting types with clustered brood belong to the same genus but the *laeviceps* group. Samples from Albay and Sorsogon which were in closed nesting type were positively identified as *T. biroi* while the open nested found in hollow trees with clustered brood were identified as *T. sapiens*. Abu Hassan Jalil (2017) reported in Chapter 10 of his book the meliponiculture activities in the Philippines as contributed by Mostoles (2017), specifically showcasing the diversity of bee species in different ecosystems and landscapes in Bicol.

Pollen Sources of Stingless Bees and Bloom Pattern

Pollen collected from plants found in the different survey areas, particularly in meliponaries is presented fully in the brochure entitled "Pollen Sources of Stingless Bees in the Bicol Region" which is the output of this research. Some of these plants, the raw and acetolyzed pollen are presented in Figures 3 and 4.

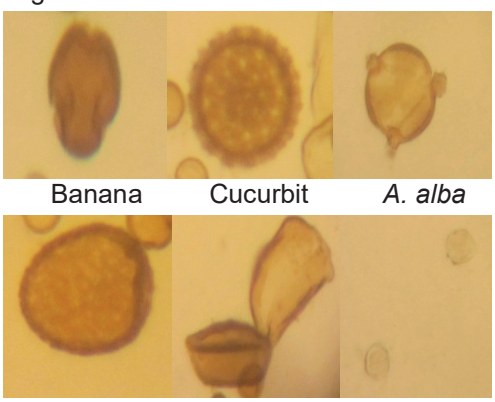

Fig. 3. Some of the acetolyzed pollen collected from meliponaries.

Camarines Sur. In four meliponaries, minor differences were observed on the kind of plants visited by the stingless bees. Bee bread obtained from the hives showed a variety of pollen foraged by the worker bees and brought inside the hives. At MTB meliponary located at San Vicente (Pamplona), the pollen sources were: coconut (*Cocos nucifera*), as most dominant, papaya (*Carica*

Coconut (*Cocos nucifera*)

Citrus (*Citrus maxima*)

Fig. 4. Plant foraged by stingless bees in the meliponaries.

papaya), banana (*Musa* sp)., Ipil-ipil (*Leucaena leucocephala),* balingbing (*Averrhoa carambola)*, lemon (*Citrus sp),* avocado (*Persia americana)*, squash (*Cucurbita maxima)*, kamias (*Hedychium coronarium)*, Lipote/ baligang (*Syzygium polycephaloides),* tubog (*Ficus nota*). At San Jose (Pili), pollen sources were banana, papaya, coconut, guava (*Psidium guajava),* pili nut (*Canarium ovatum),* acacia *(Acacia mangium)*, santan (*Ixora grandiflora)*, corn (*Zea mays)*, eggplant (*Solanum melongena),* bitter gourd (*Momordica charantia)*, kurikuri (*Wedelia biflora*), shanghai beauty, mango, lemon and palm tree (*Palmea sp.). C*olonies at Gainza had foraged pollen from coconut, santan, "mambog", acacia, and mango (*Mangifera indica*). Similar pollen was found in the bee bread from the colonies at Bula with sampaloc and pigeon pea as additional pollen sources. Other pollen types were observed but require further identification.

Albay. At Rafael's apiary at Paulog, were corn, jackfruit, coconut, kuri-kuri, squash, ampalaya, *Euphorbia*, *Mimosa pudica*, santan, legumes and eggplant. Corn and coconut were the most abundant source in the area. Palconitin's apiary at Magcasili, Guinobatan also had coconut, vegetables, and fruit crops pollen found in its bee bread. However, because the area is surrounded by coconut, most of the pollen observed in the bee bread was the coconut pollen.

Sorsogon. The PHI or Tim de los Reyes' apiary at Cumadcad, Sorsogon had coconut, mango, avocado, star apple, papaya, banana, jackfruit, and flowering plants as its sources of pollen. Examination though of the bee bread showed a dominance of coconut and avocado pollen types. At Barcelona's apiary (Pilar, Sorsogon), coconut, mahogany, gmelina , mango, narra, cacao, and pili were found in the area. However, pollen types collected from the bee bread were from coconut, narra, and mango. Other fruit-bearing trees were found in the apiary, but no indication of its pollen types was observed in the acetolyzed samples. Grajo's meliponary located at Casiguran, Sorsogon had coconut, pineapple, corn, and lemon as pollen sources of their stingless bees. Other fruit trees were likewise found in the area, but no confirmation could be made whether their pollen is being foraged by the bees.

Masbate. Stingless bees at the meliponary of Mr. Ostia at Aroroy foraged pollen from coconut, mango, chico, 'bangkal', and some mangrove plant species. Bee bread analysis showed more of the coconut, mango, and the mangrove plant pollen. Similarly, found at Mr. Servilla's meliponary at San Pascual, Burias was pollen from mangrove plant species and other crops such as coconut, corn, banana, jackfruit, and mango.

Catanduanes. The only meliponary evaluated for its pollen sources was Balmadrid's at San Andres, Catanduanes with coconut as the major pollen source of stingless bees. Other pollen types in the bee bread were acacia, golden shower, mango, banana, papaya, guyabano,

avocado, santol, mango, corn, guava, and some flowering plants. At the coastal areas of Catanduanes, coconut and vegetable crops were in bloom for three months. Baligang and santol had flowers from Jan.-Feb. while balimbing bloomed in February. Bloom pattern was limited to three months due to abiotic factors and lack of cooperation from the apiary owners to monitor the flowering plants. Year-round monitoring is recommended for comprehensive data as a guide to the beekeepers in every province.

Camarines Norte. A newly established apiary at Lorenzo Ruiz, Camarines Norte owned by Mr. Borromeo showed coconut, mango, and lemon as the major pollen sources. Based on the 3-month calendar, it was observed that in mangrove areas, six plants bloomed from December to February while only miyapi (*Avicennia rumphiana*) bloomed in February. In meliponaries located in an agroecosystem , 12 plants bloomed for three months while star apple and avocado had flowers only in February and January-February, respectively. In apiaries in upland and semi-forest areas, there were 4 plants in bloom from Dec.-Feb., narra and santol from Jan.-Feb. and mahogany in February.

Pollination of Pigeon Pea

There was a significant difference among the treatments in terms of the number of flowers pollinated by stingless bees in pigeon pea. The highest number of flower pollinated was in the cage containing 15 individuals of the stingless bee with a pollination of 96.88%, followed by open -pollination with pollinators around the area with 81.46%, 10 individuals of stingless bee in a cage with 68.81%, and 5 individuals of stingless bee in the cage with 54.23%. The lowest percent of pollination is a closed condition with no pollinator. Pollination of pigeon pea could be achieved successfully using 15 individuals of stingless bee per branch but using 5 to 10 individuals of stingless bee or with at exposure of the flower to the various pollinators (open pollination) could yield similar results. In Thailand, Anchalee Sawattum in the book edited by Abu Hassan Jalil (2017) listed ornamentals, trees and weed species which are food sources of the stingless bees. Bob Luttrell as cited by Abu Hassan Jalil (2017) identified plants and trees as sources of nectar and pollen as well as resins in various regions in South America, Mexico and North America. Stingless bees are particularly important pollinators of tropical plants, visiting approximately 90 crop species. Some habits of stingless bees resemble those of honeybees, including their preference for a wide range of crop species, making them attractive for commercial management.

Food and Cosmetic Products from Stingless Bees

Refinement of the Skin Care Product developed by the Regional Apiculture Center (RAC). The moisturizing cream developed by the RAC for the past year used a different base cream (Van Cream), added with propolis, virgin coconut oil, and scent. The product is a soft cream that is soothing to the skin, dirty white with odor being mild with the addition of a natural scent to mask the smell of the propolis. The texture is smooth and particles of the propolis are not evident. In the preparation of the moisturizing cream- a predetermined volume of the honey, propolis, wax, van cream, virgin coconut oil were mixed aseptically. The wax was first melted in a beaker with the propolis to allow the ethanol to evaporate. This was allowed to cool down then mixed with the van cream with continuous stirring. Several drops of honey and virgin coconut oil were added with stirring. A scent was likewise added for the moisturizing cream. Once the materials were thoroughly mixed, these were transferred to a 5 g container using a small spatula, covered, and then

labelled. The developed moisturizing cream was evaluated for its physical attributes particularly the color, odor, and consistency. Improving the cream made use of pure propolis extract replacing the ethanolic extract. Using the pure extract made it easier to mix thoroughly the propolis with the van cream. Instead of using VCO, the oil added to the cream was Pili Pulp Oil (PPO) which is a product in the region. No scent was used. Just like the previous preparation, the color of the cream is cream/dirty white due to the propolis. This is however slightly lighter in color compared with the previous mixture. The smell of the PPO is distinct giving it a scent of pili nut.

The moisturizing cream was tested on the skin. By 15 individuals used for a period of six months but was not compared with any control. They claimed that skin irritations were easily eased with a slight removal of scars from wounds. Likewise, some who used it for their face with a growing pimples said that applying the cream prevented further pimple to develop.

Other new food products using stingless bees as an ingredient are presented in Figure 5 and discussed below:

Macaroons with honey. Macaroons are a popular sweet food product basically from desiccated coconut. The tangy honey from stingless bees was used as a substitute for refined sugar to prolong the shelf life. Normally, the shelf life of macaroons is for only 3 days under ambient temperature and a week when refrigerated. Macaroons cooked using honey instead of refined sugar was found to be less sweet, based on the testimonies from the consumers. Reduced sugar substituted with honey in macaroons is preferred by consumers particularly the diabetics. Under ambient temperature, the texture of the macaroons remained the same after 10 days which can further be stored under the refrigerated condition for more than 2 weeks. Macaroon with honey is packed in small polypropylene plastics (4 pcs) and in a carton/box with 12 pcs. It could be packed in small plastic (hard) containers. A benefit that extended shelf life and the nutrients in using the tangy honey of stingless bees.

***Polvoron with pollen*.** Stingless bees' pollen is high in protein which could be given to malnourished children. However, the bee bread obtained from the hives of stingless bees after processing is not aesthetically acceptable to children and even the other age groups. Its granulated form taken directly from the hive is more often associated with animal food. Considering the nutritional benefits of the bee bread (pollen) from stingless bees, adding this to polvoron will be more palatable to the children. Particles of the bee bread were powdered and added as an ingredient in making polvoron. The proportion of which was first determined before mass production. A few drops of stingless bees' honey was used to reduce the amount of sugar granules which is used to bind the flour. The polvoron was packed in colored plastic and small plastic polyethylene bag. Sensory evaluation of the product was done and results showed preference and shelf life of polvoron extended to 26 days. Succeeding production had its size doubled with a corresponding increase in the price.

***Honey-Propolis Candies*.** Honey is a natural sweetener while propolis has antifungal and antibacterial properties. Thus, these were used in making the honey-propolis candies. The ingredients used in preparing the candies were: honey, propolis, sugar, and condensed milk of determined proportions. The mixture was cut based on the desired shape and then allowed to harden. The candies were packed in small PEBs. Several trials of the candy produced a chewable type but shelf life was too short. Individuals who ate the candy said that it relieved their throat and reduced odor from their

mouths.

Alco - propolis Sanitizer. With propolis having both antibacterial and antifungal properties, a cleansing product was done with the mixture of alcohol and propolis of predetermined proportion. This can be used to cleanse the hands or any part of the body which comes handy in the plastic container.

Propolis Spray. The propolis spray was formulated to be used as a throat spray. It is sprayed directly into the mouth to relieve the throat. It is composed of a predetermined proportion of ethyl alcohol and propolis. The spray was also used as astringent for wounds, skin rashes, insect bites/ venoms, boil, pimples, and other skin irregularities. . This is quite extensive since the materials used such as the propolis is expensive and the end product is the extracted propolis itself without any additive.

Bath Soap. The bath soap developed is a combination of oils and honey and propolis as additives. The standard method and ingredients in making a bath soap were followed but honey and propolis were added. These were moulded in a carton/plastic cells. The preparation was allowed to cure for a minimum of 3 weeks and the products were packed in small polyethylene bags . Several persons were requested to use the bath soap for sensory analysis. The bath soap was evaluated based on the lather, odor, moisturizing effect, color, and germicidal effect. In terms of lather, the respondents found it to be acceptable, for the odor some claimed it to be acceptable, but others rated it as slightly acceptable. For the color, moisturizing and germicidal effect, these were rated as acceptable in more than 50% of the respondents. Among the parameters rated, the odor was found to be less acceptable to the respondents. The overall effect, the product is acceptable. The products were sold to interested individuals and there was no negative feedback received.

Shampoo Bar. This product was formulated with the combination of different oils, glycerin, lye, guava wine, and propolis. The mixture was meld in cells made of thick boards. The shampoo bar can be dissolved in water to produce a liquid shampoo. The soap was claimed by one user that it helped control head louse, especially in children. In a recent training conducted at Sanchez-Mira, Cagayan, this product was promoted for such use. Shown in Figure 5 are the products developed by the researchers.

Cosmetic products

Food products

Fig.5. Cosmetic and Food Products.

Three (3) IEC materials were prepared on the following: Distribution and abundance of stingless bees in Bicol, Food and Cosmetic products from stingless bees, and Pollen Sources of Stingless Bees in the Bicol region (Fig.6). Though copies are

limited, these have been used in trainings, exhibits, and seminars/fora to encourage more individuals to venture into beekeeping using stingless bees. Likewise, these materials are also useful to students working on research on stingless bees.

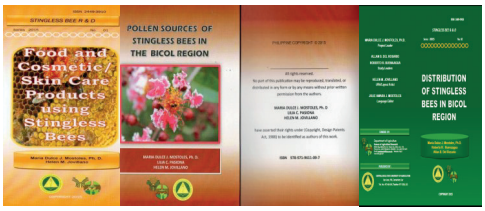

Fig. 6. IEC materials developed and utilized during trainings.

CONCLUSION

Positive occurrences of stingless bee colonies in the six (6) provinces in the Bicol region. These were sighted in Albay (Barangays of Dinoronan and De la Paz, Daraga), Camarines Norte (Barangay Matacong, Brgy. Dagotdotan, and Brgy. Mampurong, of San Lorenzo Ruiz), Camarines Sur (Barangay of San Pedro-Aroro, Goa and Sta. Teresita Iriga City), Catanduanes (Brgy. Quezon of Bagamanoc, Brgy. San Miguel of Panganiban and Brgy. Anannong and Ugbong of Viga), Sorsogon (Brgy. San Roque of Bulusan, Brgy. Del Rosario of Pilar, Brgy, Quidolog of Prieto Diaz and Brgy. Inlagadian and Brgy. Tigbao of Casiguran), Masbate (Brgy. Matalang-Talang, Brgy. Cabangcalan and Brgy. Pinanaan of Aroroy). Open nesting with clustered brood types and closed nesting with spherical brood types of colonies were collected which belongs to the genus *Tetragonula*, groups *laeviceps*, and *carbonaria*, respectively. Sightings of the colonies were mapped for its distribution per province. Mean distances between colonies were shortest in Albay (0.39km) indicating abundance while farthest in Sorsogon (28.993km), thus declared as least abundant for stingless bee colonies.

Pollen sources of stingless bees in the meliponaries surveyed were: In Camarines Sur: coconut (*Cocos nucifera*), papaya (*Carica papaya),* banana (*Musa* sp.), Ipil-ipil (*Leucaena glauca),* balingbing (*Averrhoa carambola)*, lemon (*Citrus sp),* avocado (*Persia americana)*, squash (*Cucurbita maxima)*, kamias (*Hedychium coronarim)*, Lipote (baligang) (*Syzygium polycephaloides),* guava (*Psidium guajava),* pilinut (*Canarium ovatum),* acacia (Acacia *mangium)*, santan (*Ixora grandiflora)*, corn (*Zea mays)*, eggplant (*Solanum melongena),* bitter gourd (*Momordica charantia)*, kuri-kuri (*Wedelia biflora*), shanghai beauty, mango, lemon, palm tree (*Palmea sp.),* santan, and "mambog"; Albay: corn, jackfruit, coconut, kuri-kuri, squash, ampalaya, *Euphorbia*, *Mimosa pudica*, santan, legumes and eggplant; Sorsogon : coconut, mango, avocado, star apple, papaya, banana, jackfruit, flowering plants, mahogany, gmelina , mango, narra, cacao, pili, pineapple and lemon; Masbate: coconut, mango, chico, 'bangkal', mangrove plant species, corn, banana and jackfruit; Catanduanes: coconut, acacia, golden shower, mango, banana, papaya, guyabano, avocado, santol, mango, corn, guava and some flowering plants were the pollen sources; Camarines Norte: coconut, mango and lemon. Meliponaries in all the provinces had their stingless bees found to forage on coconut plus other horticultural, agronomic, forest, and fruit crops and mangrove plant species. Stingless bees were found to be not host-specific and its floral preferences are diverse. The majority of the plants were in bloom in the apiaries from December to February except for some trees which were only at the flowering stage starting January.

Pollination of pigeon pea using 15 individuals of stingless bee in cages significantly differed with 96.88% while 67.36% for closed pollination.

Stingless bee products such as pollen, propolis, and honey could be sold as raw materials but could be used in developing value-adding food and cosmetic products. Refinement of the moisturizing cream was done with the substitution of VCO with pili pulp oil, improved packaging, and labelling.

Incorporation of bee products on food such as macaroons with honey, polvoron with pollen, and honey-propolis candies was found acceptable to the consumers including the packaging and labelling.

Formulated and developed cosmetic products using stingless bee products were: alco-propolis sanitizer and propolis throat spray, bath soap, and shampoo bar with propolis. All of the products developed were found to be acceptable.

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MYCELIAL GROWTH PERFORMANCE OF PADDY STRAW MUSHROOM (V*olvariella volvacea*) IN BANANA WASTES

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Abstract — Saba banana (*Musa balbasiana*) is a nutritious and the most popular banana in the Philippine market. Once utilized, its fruit generates a huge amount of agricultural waste. On the other hand, the paddy straw mushroom (*Volvariella volvacea*) was known as one of the most cultivated mushrooms in the world due to its nutrients and pleasant flavor. This study aimed to determine the paddy straw mushroom mycelial production in banana (peeling, leaves, and pseudostem) wastes media. Banana wastes media were prepared by decocting banana parts tap water to produce 50% and 100% concentrations of decoction, adding 20 g of white gulaman bar and 10 g of white table sugar until the homogenous solution was attained; pH was adjusted into pH 8 using NaOH and HCl. For sterilization, the media was autoclaved for 20 minutes at 121°C /15 psi. Mycelial growth and density of *V.volvacea* were evaluated in different banana wastes media namely: T1: Coconut water, T2: Decocted Banana Peeling (50%), T3: Decocted Banana Peeling (100%), T4: Decocted Banana Leaves (50%), T5: Decocted Banana Leaves (100%), T6: Decocted Banana Pseudo stem (50%) and T7: Decocted Banana Pseudo stem (100%). Data was analysed using the Completely Randomized Design. At an average temperature of 28.6°C and 74.77% relative humidity, paddy straw mushrooms showed the highest mycelial run in T4 compared to other banana wastes media. The T2 and T3 were noted with the thickest mycelia among all evaluated media. T4 was recorded with the shortest period of ramification within 3 days and was considered as the ideal culture media for paddy straw mushroom among banana wastes media since the faster the mycelial grows, the quicker the mushroom production. Among the media tested, the T4- 50% of banana leaves extract is the ideal alternative media for *V. volvacea* mycelial production.

Keywords — ***Musa balbasiana*****, mycelial density, mycelial growth performance, *Volvariella volvacea.***

INTRODUCTION

Mushroom cultivation is a very reliable and profitable option to alleviate hunger and malnutrition in the world (Rashid et al., 2016). Various regions, particularly America, Central Europe, and South-East Asia are known to cultivate different species of mushrooms that are used as sources of food rich in vitamins and minerals utilized by the human body. Due to its known benefits both to humans and the environment, production levels of these saprophytes radically increased over the years. The growing awareness for nutritive and quality foods in the health-conscious world population has resulted in the demand for the mushroom to rapidly rise, with its cultivation practice growing worldwide.

Volvariella volvacea, also known as paddy straw mushroom is one species of edible macrofungi that are considered the third most important cultivated mushroom in the world due to its pleasant flavor and taste (Thiribhuvanamala et al., 2012). As stated by Huo et al. (2017), the market demand for *V. volvacea* has rapidly increased because of its unique flavor and texture prompting markets from all over the world to use it as a fresh dietary mushroom. Along with its unique culinary properties, this particular species is known to contain high levels of nutrients and minerals necessary for a balanced diet in humans. For instance, its protein and fat contents range between 25.9–29.6% and 2.24–3.6% per 100 g of dried fruiting bodies of *V. volvacea* (Hung et al. 2010). Additionally, it is rich in amino acids and fiber, and vitamin C content is significantly higher than that of many vegetables and fruits (Han, 2005).

However, problems in cultivating *V. volvacea* are associated with the practice and materials used. Like other species of mushroom, *V. volvacea* requires a specific set of materials and nutrients to support its growth. As reported by Bao et al. (2013), the biological efficiency of *V. volvacea* is only 15% on straw-based substrates and 30–40% on cotton-waste composts. As stated by Ukoima et al. (2009), *V. volvacea* performs a high mycelial growth on fibrous plant extract culture media. Organic materials containing a high amount of holocellulose, cellulose, and fiber are recognized as materials that support the growth and development of the genus *Volvariella.* Taking all of these into account, this study aims to promote the usage of banana wastes in cultivating *V. volvacea.*

Banana is considered one of the most important fruits in the world. In the Philippines, saba banana (*Musa balbasiana)* is the most popular cultivar grown domestically which accounts for 39% of total banana production (Castillo et al., 2015). In the Bicol region, Camarines Sur is known as the top banana producer with a 44.95% share of the region's total production (Philippine Statistics Authority, 2018). De Castro and Arenillo (2016), states that the average annual yield of the Philippines in saba banana is 9.4 ton/ha while big plantations produce about 40 ton/ha. As part of the Filipino diet, these bananas constitute a large part of the local cuisines of the country. Various dishes and desserts are known to use saba banana as an active ingredient due to its high availability and cheap price.

Even with their high economic and nutritional value, these bananas also produce a large quantity of agricultural waste that can be utilized for various biological and ecological endeavors. These agricultural wastes can be considered with significant potential for mushroom cultivation. While viewed as waste, these banana parts contain materials and nutrients that are needed by mushrooms for proper growth and development. This study aims to determine the efficiency of various banana parts including banana peelings, leaves, and pseudostems in

the production of paddy straw mushroom to improve yield and overcome the gap of malnutrition as its cultivation will provide a cheap source of nutrients and minerals needed by mankind.

MATERIALS AND METHODS

Source of Materials

The tissue culture of paddy straw mushroom was obtained from the Laboratory of Central Bicol State University of Agriculture-Sipocot Campus, Impig, Sipocot, Camarines Sur. The *V. volvacea* was cultured in a coconut water culture media and incubated for 7 days to allow mycelial growth. The cultures served as an inoculant in the evaluation of the media.

On the other hand, banana peels were gathered in front of CBSUA-Sipocot Campus from the sidewalk vendors of staple banana cuisine. Then, banana leaves and pseudostem were collected in the vicinity of CBSUA -Sipocot. Banana wastes were taken after the harvest of banana fruits.

Materials Used

This study used banana peeling, banana leaves, and banana pseudostem for culture media production along with coconut water as control media. One liter of tap water was used as a base for banana wastes decoction.

Preparation of Culture Media

Five hundred grams (for 50%) and one thousand grams (for 100%) of banana leaves, pseudostem, and banana peeling (ripe) were boiled for 10 minutes in one liter of tap water. Then, it was strained with clean cheesecloth to remove impurities. Subsequently, it was volume up to attain one liter of solution. Afterward, 20 grams of white gelatin bar and 10 grams of white table sugar were added to the solution. It was boiled until the solution was homogenized. Then each solution was placed in a clean Erlenmeyer flask, sealed with a cotton plug and secured with aluminum foil. Before sterilization, the pH was adjusted to pH 8 since, according to Reyes et al. (1998), paddy straw mushroom grows luxuriantly at pH 8 under dark and sealed conditions. Finally, it was autoclaved for 20 min at 121°C /15 psi.

Also, one liter of matured coconut water was added with 20 g of white gulaman bar. It was boiled until a homogenized solution was attained. Then it was placed in a clean Erlenmeyer flask, sealed with a cotton plug and aluminum foil. Prior to sterilization, pH was adjusted at pH 8. Then it was sterilized for 20 min at 121°C/ 15 psi. Coconut water was not added with white table sugar since according to Santoso et al. (1999), matured coconut water contains 92 % sucrose which makes it suitable for mycelial cultivation. Also, it was used as the control for the study of Bellere et al. (2017).

Plating and Inoculation of Paddy Straw Mushroom

The newly sterilized media was dispensed in a sterilized Petri plate until it solidifies. Then, using a sterile cork borer, seven days old 10 mm mycelial block was inoculated at the center of the media with three replications each. Subsequently, it was sealed with cling wrap.

Incubation and Ramification of Paddy Straw Mushroom Culture

The newly inoculated media was stored in a dark cabinet at room temperature since according to Sahoo (2014), *V. volvacea* grows well in dark conditions. The culture media was incubated until it was fully ramified.

Evaluation of Mycelial Growth Performance of Paddy Straw Mushroom in Culture Media

The mycelial growth performance of paddy straw mushroom in banana waste media was evaluated through its mycelial increment, thickness, and incubation period observed daily along with the temperature and relative humidity every 7:00 am, 12:00 pm, and 5:00 pm. The mycelial increment was determined by measuring the continuous growth of mycelia until it fully ramified the media. Its thickness and incubation period were observed to identify the ideal banana waste culture media. The gathered data were evaluated statistically using T-test and Analysis of Variance (ANOVA) and were presented separately according to the concentration.

Experimental Design

Completely Randomized Design (CRD) was employed in setting up the treatment that was replicated thrice. The treatments are:

T1- 1L CW + 20g GB
T2- 500g BP + 10g WTS + 20g GB + 1L TW
T3- 1000g BP + 10g WTS + 20g GB + 1L TW
T4- 500g BL + 10g WTS + 20g GB + 1L TW
T5- 1000g BL + 10g WTS + 20g GB + 1L TW
T6- 500g BPs + 10g WTS + 20g GB + 1L TW
T7- 1000 BPs + 10g WTS + 20g GB + 1L TW

Note: CW – Coconut Water, BP – Banana Peel, BL- Banana Leaves, BPs- Banana Pseudostem, WTS- White Table Sugar, GB- Gulaman Bar, TW- Tap Water

RESULTS AND DISCUSSION

Mycelial Growth Performance of Paddy Straw Mushroom

Mycelia refer to the vegetative part of a fungus which consists of a mass of branching and thread-like hyphae (Bellere et al., 2017). The nutritional content of the media can affect the growth of mycelia since according to Liebig's Law of Minimum if any of the required nutrients was unavailable, the organism cannot reach its full potential.

At 100% concentration, the first day of observation CW recorded with the highest mycelial run of 13.08 mm. Followed by BL (T5) with 6.08 mm, 2.58 mm of BPs (T7), and BP (T3) with the lowest mycelial run of 2.25 mm. On the second day, CW (T1) has shown the highest mycelial run of 15.34 mm compared to the BL (T5) with 9.25 mm, 6.09 mm of BPs (T7), and BP (T3) with the lowest mycelial run of 3.83 mm. During the third day of incubation, paddy straw mushroom has performed the highest mycelial increment to the CW (T1) with 10.5 mm, followed by BL (T5), BP (T3), and lastly BPs (T7) with 10.34 mm, 6.92 mm, and 3.75 mm respectively. In considering the mycelial density, the BP (T3) was recorded with the thickest mycelia from the first to the third day of observation compared to the other media.

Table 1. Mycelial Growth Increment of Paddy Straw Mushroom (100%).

Treatment	Day 1		Day 2		Day 3	
	Mean	MD	Mean	MD	Mean	MD
T1	13.08	+	15.34	++	10.5	++
T3	2.25	++	3.83	+++	6.92	+++
T5	6.08	+	9.25	+	10.34	+
T7	2.58	+	6.09	+	3.75	+

Note: MD stands for Mycelial Density and the lowest degree marked as + of mycelial thickness, intermediate degree marked as ++, and the highest degree marked as +++ of mycelial thickness (Razak et al., 2012)

During the incubation period, CW has shown the fastest mycelial run among other media. The ability of the coconut water to stimulate fast mycelial growth of paddy straw mushroom can be attributed to its high sugar content since according to Santoso et al. (1999), it contains 92% of sucrose making it suitable for cultivating mycelia. In considering the mycelial density, BP 100% has the thickest mycelia among others which can be associated with its high concentration of Potassium, Manganese, Calcium, and Iron (Anhwange, 2009). The banana peeling's high nutritive value could be the reason for it to sustain the mycelial run.

Table 2. Mycelial Growth Increment of Paddy Straw Mushroom in (50%).

Treatment	Day 1		Day 2		Day 3	
	Mean (mm)	MD	Mean (mm)	MD	Mean (mm)	MD
T2	4.58	++	5.17	+++	11.17	+++
T4	10.42	+	13.25	+	14.33	+
T6	4.67	+	6.58	+	12	+

Note: MD stands for Mycelial Density and the lowest degree marked as + of mycelial thickness, intermediate degree marked as ++, and the highest degree marked as +++ of mycelial thickness (Razak et al., 2012)

On the other hand, during the first day of observation BL (T4) had the highest mycelial run of 10.42 mm. The BPs (T6) followed by 4.67 mm and with the lowest mycelial run of 4.58 mm the BP (T2). On the second day of incubation, the BL (T4) was noted with the highest mycelial run of 13.25 mm. Then, BPs (T6) followed with 6.58 mm and BP (T2) with the lowest mycelial run of 5.17 mm. On the third day, the BL (T4) remained with the highest mycelial run of 14.33 mm followed by BPs (T6) with 12 mm while BP was noted with the lowest mycelial run of 11.17 mm. In the comparison of mycelial density, BP (T3) has the thickest mycelia among other media.

The BL (T4) had the highest mycelial run from first to the third day of incubation. The capacity of banana leaves to sustain mycelial growth to paddy straw mushroom can be attributed to its distinctive nutritional content of cellulose, lignin, starch, and lipophilic extractives in leaf sheaths (Oliviera et al., 2007). Additionally, Rahman (2014, claimed that it contains cellulose, ash, and pentose sugar on the banana stalk. The presence of these chemicals can be the reason for the fast mycelial run of paddy straw mushroom in banana leaves.

Meanwhile, shown in Table 3, the CW (T1) was recorded with the shortest period of incubation within three days among treatments. While the BL (T5), BP (T3), and lastly BPs (T7) were fully colonized within 4, 5, and 11 days respectively. The short period of a full ramification of *V. volvacea* on coconut water media can be attributed to its high sugar content (Santoso et al., 1999).

Table 3. Number of Incubation Period of Paddy Straw Mushroom in Banana Wastes Media (100%).

Treatment	Number of Days of Full Ramification
T1 – Matured Coconut Water	3
T3 – Banana Peel (100%)	5
T5 – Banana Leaves (100%)	4
T7 – Banana Pseudo stem (100%)	11

The data in Table 4 revealed that the Coconut Water (T1), and BL (T4) have a shorter period of incubation within three days while the BP (T2) and BPs (T6) were fully ramifies for five days.

Table 4. Number of Incubation Period of Paddy Straw Mushroom in Banana Wastes Media (50 %)

Treatment	No. of Days of Full Ramification
T1- Coconut Water	3
T2 – Banana Peel (50%)	5
T4 – Banana Leaves (50%)	3
T6 – Banana Pseudo stem (50%)	5

The fast colonization of *V. volvacea* in coconut water and banana leaves can be associated to the nutritional content of banana leaves since it contains higher amount of cellulose, lignin (Oliviera et al., 2007) and sugar (Rahman, 2014) that can support mycelial growth.

Table 5. Summary of the Significant Difference on Daily Mycelial Run of Paddy Straw Mushroom in Banana Wastes Media (100%).

No. of Days	F-Test Value	Decision	Interpretation
Day 1	0.002	Accept	No Significance
Day 2	0.002	Accept	No Significance
Day 3	0.025	Accept	No Significance

Level of Significance= .05
Critical F-test Value = 4.07

Based on the data showed in table 6, the daily mycelial run was found to be not significantly different since the computed F-test value of 0.002 for Day 1, 0.002 for Day 2 and 0.025 for Day 3 showed that it is lesser than the critical F-value of 4.07.

Subsequently, the computed F-test value of daily mycelial run of paddy straw mushroom in banana wastes media with 100% concentration was found to be lesser than the critical F-value. This means that the null hypothesis was accepted that there is no significant difference in the daily mycelial run of paddy straw mushroom in a 100% concentration of banana wastes media.

Therefore, the data proposed that the banana wastes media in 100% concentration can be used as an alternative culture media for paddy straw mushroom production.

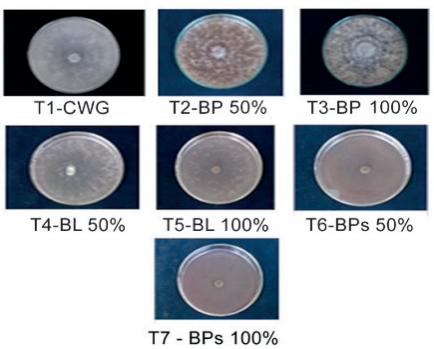

Fig.1. Fully ramified banana waste culture media of paddy straw Mushroom.

Table 6. Summary of the Significant Difference on Daily Mycelial Run of Paddy Straw Mushroom in Banana Wastes Media (50%**).**

No. of Days	F-Test Value	Decision	Interpretation
Day 1	0.02	Accept	No Significance
Day 2	0.09	Accept	No Significance
Day 3	0.61	Accept	No Significance

Level of Significance =0.05
Critical F-test Value=5.14

It was shown in the Table 6 that the daily mycelial run of paddy straw mushroom in banana wastes media with 50% concentration was not significantly different based from the computed F-Test value of 0.02, 0.09, and 0.61 for Day 1, Day 2, and Day 3 respectively wherein it was noted to be lesser than the critical F-value of 5.14.

The computed F-Test value of the daily mycelial run was found lesser than the critical F-value. Thus, the null hypothesis was accepted that is there is no significant difference in the mycelial run of paddy straw mushroom in 50% concentration of banana wastes media. Thus, these findings suggest that the banana wastes media in 50% concentration can be utilized as an alternative culture media for paddy straw mushroom production.

CONCLUSION

The study elucidated that paddy straw mushroom grows at an average temperature of 28.6°C and 74.8 % relative humidity. Among all banana waste media, banana leaves (100% and 50% concentration) have shown the highest mycelial increment within three days of observation. Additionally, BP (T2) and BP (T3) solution showed the thickest mycelial density as compared with the rest of the banana waste media that elucidated thin mycelial density, likewise, both BL (T5) and BL (T4) generates the shortest days of incubation. Therefore, banana leaves were considered as an ideal culture media for paddy straw mushroom among the evaluated banana wastes in terms of the number of days of incubation. Moreover, evaluated *saba* banana parts can be utilized as an alternative culture media for paddy straw mushroom production.

Some recommendations have been crafted; adjusting the range of temperature within 30-35°C for favorable temperature for paddy straw mushroom during incubation, utilize other variety of banana for cultivating paddy straw mushroom, utilize saba (*Musa balbasiana*) banana leaves as a culture media in the cultivation and production of other varieties of mushroom, and use a mixture of saba banana parts with high amounts of nutrients in producing alternative culture media for cultivating paddy straw mushroom.

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EFFECTS OF ORGANIC SOIL AMENDMENTS FOR GROWTH, YIELD, AND FRUIT CONTENTS OF HOT PEPPER *(Capsicum frutescens* L.*)*

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Abstract — Hot pepper is a widely-used vegetable and spice crop due to its flavor and pungent taste, rich nutritive value, and medicinal uses. Its capsaicinoids content, which caused the pungency, is known to have anti-inflammatory, anti-oxidant, and medicinal properties. A field experiment evaluated the growth, yield, and fruit quality of organically grown hot pepper Tinghala variety. It was laid-out using Randomized Complete Block Design with twelve treatments and three replications of soil amendments with organic sources. The results indicated that growth variations were significant ($p<0.05$) at different periods with the effects of VC+Calphos+FFJ, VC+FPJ, and VC significantly highest at the early stages of plant development. The number of days to flowering and percent fruit set varied significantly, but the days to fruit set remained the same. The different fertilization caused significant effects on the yield performance of hot pepper ($p<0.05$), with the VC+Calphos providing the highest in the total number of fruits per plant, total weight, and economic yield. The capsaicinoid content in fruits was highest in VC+Calphos+FFJ but, the differences across treatments were insignificant. The nitrogen, organic matter, and pH contents of soil slightly improved while potassium decreased. Phosphorus significantly increased with organic fertilization. Organic soil amendments, therefore, can be a viable option for hot pepper production.

Keywords **— Capsaicinoids content, *Capsicum frutescens*, fruit quality, organic soil amendments, soil analysis.**

INTRODUCTION

Organic soil amendments are organic materials added to the soil to enhance its properties and to provide nourishment for plants' growth and development. It includes all products such as organic fertilizers, composts or soil conditioners, microbial inoculants, and organic plant supplements (Bureau of Agriculture and Fisheries Standards, 2016).

As modern agriculture dominates agricultural production through high yields using chemical fertilizers, it is not sustainable and creates an unfavorable impact on the environment and consumers' health. The use of the environment and health-friendly organic fertilizers is advocated today as new insights to achieve sufficient and sustainable yields and to meet the increasing global demands for food and agricultural products (Ertani *et al.*, 2015). Inorganic fertilization, commonly used included vermicompost, indigenous microorganisms, fermented plant juice, fermented fruit juice, calphos, oriental herbal nutrients, and many others (Zamora *et al.*, 2016).

Capsicum is an economically important, widely consumed vegetable and spice crop throughout the world (Hegde *et al*., 2014; Saleh *et al*., 2018; Jarret *et al*., 2019; Batiha *et al*., 2020). *Capsicum* spp. contain several bioactive compounds with nutritional and pharmaceutical importance varying between pepper types (Olatunji, 2020). Many species of *Capsicum* are being cultivated worldwide as a commercial crop for their economic value (Reddy *et al*., 2016).

Capsicum frutescens L. is a species utilized and studied for its capsaicinoids, the alkaloid compounds responsible for the pungency of *Capsicum* species (Hamed *et al*., 2019). The crop has a promising potential given its phytochemical, nutritive, medicinal, and economic value.

The interest in the production and use of hot pepper is increasing. In the locale of the study, some farmers are growing hot peppers both for family consumption and for commercial purposes. To establish organic production of hot pepper and to improve yield and fruit quality, studies on the use of locally available materials as organic soil amendments would provide solutions to constraints on productivity and food safety while determining the crop's performance, specifically, on the effect on growth, yield, and fruit quality; alkaloid content of fruits, and; macronutrient content of the soil after production.

MATERIALS AND METHODS

Experimental Site

The study was conducted at San Jose, Camarines Sur, situated at the southern part of the Province, with coordinates 13º 42' North and 123º 31' East, 30.4 meters of elevation above mean sea level (PhilAtlas, 2020).

The Tinghala variety of hot pepper was used as the test crop in the experiment.

Experimental Design and Treatments

The experiment was laid out in a Randomized Complete Block Design (RCBD) with 12 treatments and three replicates: T1- Recommended Rate of Inorganic fertilization (RRI), T2- vermicompost (VC), T3-Calphos, T4- fermented plant juice (FPJ) of water spinach, T5- fermented fruit juice (FFJ) banana, T6- VC + Calphos, T7- VC + FPJ, T8- VC + FFJ, T9- Calphos + FPJ, T10- Calphos + FFJ, T11- VC + Calphos + FPJ and T12- VC + Calphos + FFJ.

Each block had 12 plots, measuring 410 cm x 420 cm. Each plot had 42 plants arranged in six rows with seven hills in a row. Ten plants were used as samples for the parameters observed.

Experimental Procedures

Soil Sampling and Analysis

Soil samples were collected from nine sites in the experimental area following an X pattern (Mondal, 2020) at about 2.5 cm thick and 5 cm wide at specified depth using a spade, mixed and collected 1 kg soil, air-dried and sieved, packed and labeled in a plastic bag. It was analyzed at the Soil Laboratory of the Department of Agriculture (DA) – Regional Field Office No. 5 at Naga City. The following methods were used in the analysis: N by modified Kjeldal method, P by Olsen method, K by ammonium acetate method (PCARRD, 1980), and pH by potentiometric method. The soil contained 0.15% Nitrogen, 16.62 ppm Phosphorus (medium), 0.14 meg/100g Potassium (deficient), 2.93% (medium) organic matter content and pH of 5.13 (slightly acidic).

Land preparation, Seedling Production, and Transplanting

The land and plot beds were prepared in October 2018 following the experimental design. Seeds were sown in seedling trays with a mixture of equal amounts of vermicompost, rice hull charcoal, and garden soil. Regular watering was done to maintain adequate moisture. Seedlings were hardened through gradual exposure to sunlight and withdrawal of water one week before transplanting. Four-week-old healthy and sturdy seedlings were transplanted in the field at a distance of 70 cm between rows and 60 cm within a row, and one seedling per hill at a depth of 5 cm, covered with soil pressing lightly near the stem to ensure maximum contact between roots and soil and for better plant establishment.

Preparation and Application of Fertilizer

Fertilizer application was based on the treatments used, and on the result of soil and nutrient analysis. The nutrient requirement of hot pepper: N - 60 kg/ha; P - 40 kg/ha, and; K - 150 kg/ha. Table 1 presents the preparation, rate, and frequency of application of the treatments used. FPJ, FFJ, and calphos were chemically analyzed at the Agricultural Systems Institute, Division of Soil Science of the University of the Philippines, College of Agriculture and Food Science at Los Baños, Laguna.

Data Collection

Growth Parameters

The growth parameters include the plant height, days to flowering, days to fruit set, and percent fruit set. Plant height was measured every fifteen days after transplanting up to the start of harvesting.

The number of days from transplanting to the first flowering was recorded when 50% of the plants flowered.

The number of days from flowering to the first fruit set was taken when 50% of the plants had set fruits.

The percent fruit set was obtained using the formula:

$$\text{Fruit Set}(\%) = \frac{\text{No. of Fruits}}{\text{Total No. of Flowers}} \times 100$$

Yield Parameters

The yield parameters were the number of fruits per plant, the weight of fruits per plant, economic yield, and harvest index. The total number of fruits harvested per plant from the ten sample plants was counted. The total weight of fruits in grams was measured. The economic yield was computed using the total weight of harvested fruits per plant and the number of plants in a hectare.

The Harvest Index was determined by using the formula:

$$\text{HI} = \frac{\text{Economic yield (kg)}}{\text{Biological Yield (kg)}} \times 100$$

Table 1. Soil amendments, the nature, and the rate or frequency of application for hot pepper plots.

Soil Amendments	Nutrient Analysis				Raw Materials Used and Preparation	Rate and Frequency of Application
	N (%)	P (%)	K (%)	Ca (%)		
Recommended Rate of Inorganic Fertilization	14.0 46.0 -	14.0 - -	14.0 - 60.0	- - -	Complete fertilizer Urea Muriate of Potash	Basal: 492 grams per plot measuring 17.22 m^2 Side dress: a mixture of 74.9 grams Urea and 315.7 grams Muriate of Potash per plot
Vermicompost	1.19	1.31	0.37	-	African Night Crawler, mud press, cow manure; Organic Agriculture Development Program (OADP) of CBSUA-Pili, Cam. Sur;	Basal: 69.8 kgs per plot
Fermented Plant Juice (water spinach)	0.33	0.13	2.0	-	Water spinach and molasses at 1:1 ratio, fermented for 14 days	6 tbsp/L H_2O; weekly application
Fermented Fruit Juice (Saba banana)	0.31	0.1	1.57	-	Fruits of Saba banana and molasses at 1:1 ratio, fermented for 14 days	6 tbsp/L H_2O; weekly application
Calphos	-	0.16	-	0.75	Eggshells, coco vinegar; a ratio of 2 kilos of eggshells to 5 gallons of coconut vinegar, fermented for 20 days	2 tbsp/L H_2O; weekly application

Alkaloid Content of Fruits

The pungency level of hot pepper fruits was determined through its alkaloid content. Fruit samples were obtained from freshly-harvested ripe hot pepper fruits in each treatment, packed in boxes, labeled, and were brought to Societe Generale de Surveillance (SGS) Philippines, Inc. Laboratory at Makati City for analysis. Capsaicinoids content of the fruits was analyzed based on the Association of Official Analytical Chemists (AOAC) Official Methods of Analysis (AOAC International & Latimer, 2012).

Soil Analysis

After crop production, soil samples per treatment were obtained and submitted to the Soil laboratory of the Department of Agriculture – Regional Field Office No. 5 at Naga City for N, P, K, pH, and OM content analyses, using the following methods: N by modified Kjeidal method, P by Olsen method, K by ammonium acetate method (PCARRD, 1980) and pH by potentiometric method.

Data Analysis

Data were analyzed using frequency count, mean, percentage, and ranking. The significant difference among treatments was analyzed through Analysis of Variance (ANOVA) and the comparison of treatment means through the Duncan Multiple Range Test (DMRT) at 5% level of probability using the Statistical Tool for Agricultural Research (International Rice Research Institute, 2013).

RESULTS AND DISCUSSION

Growth Parameters

The analysis of variance showed that treatments caused a significant ($p<0.05$) effect on plant height at a

different time of measurements. Plant height was significantly highest at T12 (23.27 cm) at 15 days after transplanting (DAT); T12 (25.07 cm) and T7 (24.90 cm) at 30 DAT; and T2 (31.97 cm) and T7 (31.77 cm) at 45 DAT. From 45 to 90 DAT, the effect of organic fertilization, other than those with combined foliar fertilizers in T9 and T10, was not significant compared with T1. Nutrients provided by vermicompost and foliar spray or their combinations have a synergistic effect on the plant growth comparable with inorganic fertilizers. The vermicompost, as an organic fertilizer provides some essential nutrients supporting plant growth compared to chemical fertilizers (Dinani *et al*., 2014), thus its application enriches the soil microorganism, plant growth and nutrient content, and shelf life of the produce (Motcha Rakkini *et al*., 2017).

A significant difference (p=0.003) among treatments was observed in the number of days to flowering. Plants in T9 (33 days), T11 (33 days), and T6 (35 days) significantly flowered earlier than plants in T4 (44 days), T10 (45 days), T2 (47 days), and T5 (49 days). T5 - FFJ Banana (49 days) flowered significantly late than the other treatments. The duration was comparable with the normal flowering period of most hot peppers at 7 – 8 weeks (Balfour, 2006). As vermicompost has been shown to promote flowering, the number and the biomass of the flowers, and the production of fruit yield (Zaefarian *et al*., 2016), the result confirmed that Calphos primarily acts in inducing flowering among plants, promoting higher yield, inducing longer shelf-life of fruits, and providing added resistance to plants against pests and harmful insects. Calcium and phosphorus provide plants with their nutrient requirement for the changeover period from growing to flowering or fruiting (Schulert, 2016).

Hot pepper plants set fruits at an average of six (6) days after flowering, ranging from five to eight days. Inorganic and organic fertilization showed no significant difference in this parameter.

The percent fruit set was significantly higher in T6 (94.33%) and all other treatments ranging from 88.33% to 92.00% than T2 (VC) with 80.33% and T5 (FFJ Banana) with 76.67% fruit set, at p=0.002. Application of foliar fertilizers, excluding FFJ Banana alone, aided in improving percent fruit set in hot pepper, providing a significantly higher fruit set from plants grown using T2 only.

Yield Parameters

The analysis of variance showed that the different treatments caused significant (p=0006) effects on the total number of fruits per plant. Hot pepper plants grown using T6 gave significantly the highest number of fruits (203 fruits) than the other treatments. Plants applied with combined vermicompost (T2) or supplemented with other foliar fertilizers (T7, T8, T11, and T12), excluding T9, were similar in the number of fruits produced. Nutrients supplied by foliar fertilizers supplement the requirement of hot pepper to support growth, flowering, and fruiting. Foliar fertilizers enhanced the performance of hot pepper in the production of the number of fruits per plant, as particularly found in T6.

Treatments varied significantly (p=0.0072) in their effects on the weight of fruits per plant. T6 (239.47 grams), T1 (186.65 grams), and T8 (174.41 grams) gave a significantly higher total weight of fruits per plant compared with T9. Other types of organic fertilization were similar in effect with conventional practice. The analysis of variance was recorded at p=0.072. Similar to the total number of fruits and weight of fruits, organic fertilization in T6 (5.64 t/ha) and T8 (4.11 t/ha) were similar with T1 (4.40 t/ha). The combined benefits provided

by the vermicompost and foliar fertilizers in the growth, flowering, fruit set, and fruit development seemed to have been optimized by hot pepper plants in T6 and T8. Foliar application only provided a supplemental source of nutrients. Hence, when only foliar fertilizer was applied, the yield was significantly reduced, as evident in T9.

The total number and weight of fruits per plant are determinants of its economic yield. The economic yield then determines the production returns that can be obtained from the crop. As cited by Hegazi *et al.* (2017) in their study in sweet pepper, the increase in the availability of Phosphorus and Potassium significantly improved both the growth and yield parameters as the number of fruits per plant, fruits fresh weight, fruit length, and fruit diameter.

The harvest index of hot pepper plants was not significant across treatments (p=0.151) with the highest obtained in T3 (96.49%), followed by T8 (67.21%) while the lowest harvest was in T9 (36.88%). Harvest index, the measurement of the yield of the crop obtained by its economic yield over biological yield, is a factor that affects crop production and acts as an efficiency indicator (AgriHunt, 2016).

Alkaloid Content of Fruits

The analysis of the pungency level of hot pepper, Tinghala variety, receiving different fertilizers showed that T12 produced the hottest fruits (13,970 SHU) while the least was in T6 (8,787 SHU).

The effect on the level of capsaicinoids of hot pepper fruits varied yet not significant. The result of the study produced fruits that fall under Medium Peppers (2,000 to 19,999 SHU) based on the characterization described in the Philippine National Standard (2018) and is comparable to Jalapeno and Serrano hot peppers (Wagonner, 2017). Aside from the genetic makeup of the plant, the amount of capsaicinoids in hot pepper fruits is also dependent on the environment, light intensity and temperature at which the plant is grown, the age of the fruit, and the position of the fruit on the plant (Popelka *et al*., 2017).

Soil Analysis

The result of the analysis was interpreted as provided by the

Table 2. Effect of different organic fertilizers on yield of hot pepper plants.

TREATMENT[1]		TOTAL NUMBER OF FRUITS PER PLANT	TOTAL WEIGHT OF FRUITS PER PLANT (grams)	ECONOMIC YIELD (t/ha)	HARVEST INDEX (%)
T1	RRI	149 b[#]	186.65 ab	4.40 ab	53.35
T2	VC	92 cd	115.29 bc	2.72 bc	54.43
T3	Calphos	86 cd	113.80 bc	2.68 bc	96.49
T4	FPJ Water Spinach	98 bcd	123.55 bc	2.91 bc	50.27
T5	FFJ Banana	93 bcd	121.91 bc	2.87 bc	59.46
T6	VC + Calphos	203 a	239.47 a	5.64 a	60.24
T7	VC + FPJ	111 bcd	135.82 bc	3.20 bc	42.05
T8	VC+ FFJ	142 bc	174.41 ab	4.11 ab	67.21
T9	Calphos +FPJ	59 d	67.26 c	1.59 c	36.88
T10	Calphos+ FFJ	85 cd	110.19 bc	2.60 bc	56.05
T11	VC+Calphos+ FPJ	113 bcd	134.87 bc	3.18 bc	56.61
T12	VC+Calphos+ FFJ	99 bcd	125.55 bc	2.96 bc	54.54
CV%		**26.24**	**30.30**	**30.28**	**34.54**
Pr(>F)		**0.0006**[***]	**0.0072**[**]	**0.0072**[**]	**0.1510**[ns]
F Value		**5.12**	**3.38**	**3.39**	**1.66**

[#]Means in a column with the same letter are not significantly different at the 5% level according to DMRT.

[1]Legend: RRI – Recommended Rate of Inorganic Fertilizer; VC – Vermicompost; Calphos – Calcium phosphate; FPJ – Fermented Plant Juice; FFJ – Fermented Fruit Juice

Table 3. Capsaicinoids content of hot pepper fruits grown using different organic fertilizers.

TREATMENT[1]	CAPSAICINOIDS CONTENT (Scoville Heat Units or SHU)[2]
T1 – RRI	10,145
T2 – VC	12,121
T3 – Calphos	10,750
T4 – FPJ	11,773
T5 – FFJ	10,149
T6 – VC + Calphos	8,787
T7 – VC + FPJ	10,253
T8 – VC + FFJ	9,098
T9 – Calphos + FPJ	12,361
T10 – Calphos + FFJ	10,171
T11 – VC + Calphos + FPJ	9,511
T12 – VC + Calphos + FFJ	13, 970

Means in a column with the same letter are not significantly different at the 5% level according to DMRT.

[1]***Legend:*** *RRI – Recommended Rate of Inorganic Fertilizer; VC – Vermicompost; Calphos – Calcium phosphate; FPJ – Fermented Plant Juice; FFJ – Fermented Fruit Juice*

[2]***SHU*** *– Determined based on the Association of Official Analytical Chemists (AOAC) Official Methods of Analysis (AOAC International & Latimer, 2012).*

Department of Agriculture Regional Field Office No. 5. For pH or soil reaction: intensely acidic, pH of 5.0; moderately acidic, pH of 5.6 to 6.0; slightly acidic, pH of 6.0 to 6.5; neutral, pH of 6.6 to 7.0, and; slightly alkaline, pH of 7.1 to 8.0.

For Nitrogen: low, with organic matter (OM) lower than 2.0%; medium, with 2.1 to 4.5%OM, and; high, with OM higher than 4.5%. For Phosphorus: low, lower than 10 ppm; medium, 10 to 20 ppm, and; high, higher than 20 ppm. Potassium is deficient when the soil contains lower than 75 ppm (0.2 meq/100g of soil) and sufficient when it contains higher than 75 ppm.

The final soil analysis showed that the variations in the N, pH, OM, and K contents of the soil were not significant at 5% level, though highest at T12 for pH (5.97) and K (0.13 meq/100g) and T7 for OM (3.71%) and N (0.185%). Comparing these results with the analysis in T1 with pH of 4.72, 3.09% OM, 0.154% N, and 0.12 meq/100g K, it indicated that organic fertilization aids in improving the soil properties.

On the other hand, P content significantly (p=0.001) increased from 16.62 ppm to 79.88 ppm in T12. Other organic treatments were not significantly different from inorganic fertilization.

Generally, organic fertilization gave a better result than inorganic fertilization, which indicates that it improves soil characteristics (Assefa and Tadisse, 2019) and will gradually aid in enhancing soil chemical properties continuous application (Li *et al.,* 2018).

For Nitrogen: low, with organic matter (OM) lower than 2.0%; medium, with 2.1 to 4.5%OM, and; high, with OM higher than 4.5%. For Phosphorus: low, lower than 10 ppm; medium, 10 to 20 ppm, and; high, higher than 20 ppm. Potassium is deficient when the soil contains lower than 75 ppm (0.2 meq/100g of soil) and sufficient when it contains higher than 75 ppm.

The final soil analysis showed that the variations in the N, pH, OM, and K contents of the soil were not significant at 5% level, though highest at T12 for pH (5.97) and K (0.13 meq/100g) and

Table 4. Effect of different organic fertilizers on the N, P, K, pH, and OM contents in soils after growing hot pepper.

TREATMENT[1]		pH (1 soil:1 water)	OM (%)	N (%)	P (ppm)	K (meq/100g)
Initial Soil Analysis		5.13	2.93 (M)	0.15	16.62 (M)[a]	0.14 (D)
Final Soil Analysis						
T1	RRI	4.72	3.09	0.154	46.45 bc	0.12
T2	VC	4.95	3.09	0.154	62.21 ab	0.12
T3	Calphos	4.75	3.01	0.150	34.55 c	0.12
T4	FPJ Water Spinach	4.78	3.04	0.152	39.43 bc	0.11
T5	FFJ Banana	4.94	3.09	0.154	44.35 bc	0.12
T6	VC + Calphos	5.07	3.21	0.160	61.93 ab	0.12
T7	VC + FPJ	5.11	3.71	0.185	80.63 a	0.11
T8	VC+ FFJ	5.23	3.17	0.158	60.52 ab	0.12
T9	Calphos +FPJ	4.88	2.89	0.144	35.21 c	0.12
T10	Calphos+ FFJ	4.95	3.04	0.152	41.21 bc	0.12
T11	VC+Calphos+ FPJ	5.13	3.17	0.159	54.66 bc	0.11
T12	VC+Calphos+ FFJ	5.97	3.61	0.180	79.88 a	0.13
CV%		8.54	11.28	11.31	23.68	10.95
Pr(>F)		0.1167^{ns}	0.2626^{ns}	0.2659^{ns}	0.0010***	0.9230^{ns}
F Value		1.80	1.35	1.34	4.72	0.43

means in a column with the same letter are not significantly different at the 5% level according to DMRT.

[1]***Legend:*** *RRI – Recommended Rate of Inorganic Fertilization; VC – Vermicompost; Calphos – Calcium phosphate; FPJ – Fermented Plant Juice; FFJ – Fermented Fruit Juice*

T7 for OM (3.71%) and N (0.185%). Comparing these results with the analysis in T1 with pH of 4.72, 3.09 % OM, 0.154% N, and 0.12 meq/100g K, it indicated that organic fertilization aids in improving the soil properties.

On the other hand, P content significantly (p=0.001) increased from 16.62 ppm to 79.88 ppm in T12. Other organic treatments were not significantly different from inorganic fertilization.

Generally, organic fertilization gave a better result than inorganic fertilization, which indicates that it improves soil characteristics (Assefa *et al.,* 2019) and will gradually aid in enhancing soil chemical properties through continuous application (Li *et al.,* 2018).

CONCLUSION

The growth and yield of hot pepper fertilized with vermicompost supplemented with either calphos, FPJ or FFJ were comparable to those applied with inorganic fertilizers. In particular, vermicompost alone, VC+calphos and VC+calphos+FFJ promoted plant height; VC+calphos+FPJ and calphos+FPJ stimulated flowering, and; VC+calphos enhanced fruit setting of hot pepper. Moreover, VC+Calphos significantly improved the yield of hot pepper. VC+Calphos+FFJ provided the best combination for high capsaicinoid content.

The use of soil amendments with organic sources in growing hot pepper, specifically VC+FPJ, increased Nitrogen, Phosphorus, and organic matter contents of soil while VC+Calphos+FFJ improved the soil's pH than the conventional practice. Both the organic and inorganic fertilization promoted uptake of Potassium by plants hence, decreased its amount in the soil. It is recommended that VC+Calphos be used as an organic soil amendment for hot pepper production, with Calphos applied as a foliar fertilizer.

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DEVELOPING ALTERNATIVE PROTOCOL IN PILI (*Canarium ovatum Engl.*) PULP OIL ISOLATION

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Abstract — The study focused on the development of alternative protocol for isolating oil from pili (*Canarium ovatum*) pulp. It looked into essential aspects of pili pulp oil isolation by investigating existing protocol being employed by pili processors such as the traditional method, DOST recommended methods, and enzymatic extraction. Determining an alternative oil isolation protocol was conducted employing description matrix analysis based on the features of each of the existing protocol.

The proposed Ramirez Alternative Protocol RPOIM (Ramirez Pili Oil Isolation Method) consists of three major processes such as extraction and fractionation, oil isolation, and oil recovery. Fermentation of pulp for 1-2 days after mechanized mixing and after extraction is a distinct feature of the newly developed method. It was pilot tested using pili pulp samples from Bicol pili varieties obtained from the Department of Agriculture Regional Office V. Results confirmed the presence of oil in pili pulp as indicated by the data obtained on the percent oil yield of the samples tested such as 35.5% for Goa variety, 11.3% for Lanuza, 3.31% for Penafrancia, 9.76% for Magnaye and 25.2% for Laysa . It likewise revealed the potential of the RPOIM as alternative protocol to be used in pili pulp oil isolation.

Keywords — **Alternative protocol, oil isolation protocol, *Canarium ovatum,* pulp, pili pulp oil.**

INTRODUCTION

Pili (*Canarium ovatum Engl.*) is one of the indigenous fruit trees in the Philippines that has great potential for development (Briones, 2002). It is widely grown in the Bicol region, or even cultivated as a backyard crop. It can be propagated by seeds, but asexual method of propagation is highly recommended for the multiplication of high-yielding mother trees.

Pili is considered a tree of hope (BCAARD, 2010) because of its many uses. Kernel as the most important part, all the other parts were also noted to produce viable products. More than ten (10) food items can be prepared from the kernel. It is rich in nutrients, containing protein, carbohydrates, and oil which is aflatoxin-free and low in free fatty acids (Business Diary, 2019). It is also a source of edible oil of excellent quality that is being used in pharmaceutical and cosmetic products and for salad dressings. The pulp is used as a nutritious food delicacy and is processed into some useful products such as oil, food, and feeds. Pili shells are made into fashionable handicraft items. The tree is suited for reforestation because it makes an excellent windbreak. Its bark is tapped for its resin which is noted to be an excellent source of elemi oil, varnish, lacquer, and adhesives.

Pili is a priority commodity in the Bicol Region. It is one of the region's major products aside from coconut and abaca. A lot of researches had been conducted on the production, management, as well as the product development of pili which yielded a lot of benefits to the Bicolano farmers and entrepreneurs. Being able to further explore the pili potentials will be a promising initiative that can help alleviate the economic status of the region. This research on pulp oil isolation of pili will surely contribute knowledge on the utilization of this important resource.

The presence of oil from pili pulp has long been known. Many uses were associated with pili pulp oil in nutraceuticals and food industries (Pham and Dumandan, 2015). It was found to contain high quantities of unsaponifiable matter that contains lipid molecular species such as carotenoids and tocopherols. The potentials of the oil in the production of cosmetic products are also established due to the presence of tocopherols which are among the compounds associated with vitamin E. (Healthline, 2017). Growing demand for lipids with desirable characteristics is likewise noted (Pham and Pham, 2012).

The market potential of this natural resource from a would-be wasted material after processing the kernel into delicacies is high due to its important use in industry. Its quality which is comparable to olive oil (Pham and Pham, 2012) adds to its potential for commercialization. It can be used as a material in the manufacture of sardines, salad dressing, and many other food preparations to substitute for the imported olive oil.

The research undertaking looked into important aspects of pili pulp oil isolation. It determined the existing methods employed in pili pulp oil isolation and generate an innovative or alternative method to use. It also involved testing of the new method. Having an alternative method of extracting oil from pili pulp that is community-based and farmer-friendly may promote pili pulp oil isolation among the farmers and thus provide another income-generating opportunity for the marginalized sector.

The knowledge and technology that could be generated will be a strong support to nutraceutical and food industries and may promote economic development among pili farmers in the region. With the new knowledge generated on pili pulp oil isolation, new researches and entrepreneurial undertakings may be conceptualized. It

can serve as the basis of new research undertakings in pili product development as well as value-adding to pili by-products which may lead to the promotion of pili pulp oil technologies. It will likewise support the wise utilization of pulp which is generally thrown by households after separating it from the nut and the kernel.

MATERIALS AND METHODS

Methods employed in establishing a protocol for pili pulp oil isolation include simple steps such as a survey to determine the existing pili pulp oil extraction method; analysis of data through a description matrix analysis; developing the protocol; and validation and testing.

Determination of Existing Pili Pulp Oil Extraction Method

Benchmarking on the traditional methods employed by processors involved in pili pulp oil extraction in the region was made by interviewing pili processors. Web quest was likewise employed to determine other methods introduced by researchers and other sectors involved in pili pulp oil isolation. A description of the features of the isolation methods was noted.

Data Analysis and Development of Alternative Isolation Protocol

Description Matrix Analysis was employed in data analysis. The description and features of each protocol were presented in a matrix that served as the basis for deducing the alternative protocol. Descriptive analysis characterizes a phenomenon to be able to answer questions, identify and describe trends and variation, and create new measures of critical aspects (Loeb et al., 2017). The method of analysis was found relevant and appropriate to the data at hand.

Validation and Testing of the Alternative Protocol

The new protocol conceptualized after considering the various methods employed by pili pulp oil extractors was tested to validate its utility in the process. Its potential as an alternative method to be used was determined by using it in isolating pili pulp samples from certain pili varieties obtained from the Department of Agriculture Regional Office V (DA-ROV). It consists of the following steps.

1. Securing and Preparation of Pili Fruit Samples – Pili pulp from sample varieties obtained from DA ROV such as Goa variety, Lanuza, Penafrancia, Magnaye, Laysa and Sorsogon variety was prepared for use in the oil isolation process. These varieties are noted accessions of DA and are the ones available at the time of the coordination.
2. Pulp Oil Isolation - Samples of pili pulp were used in oil isolation using the Alternative method developed from the existing methods. The volume of recovered oil and the percent yield were noted, and the efficacy of the alternative protocol was established.

RESULTS AND DISCUSSION

The study developed an alternative protocol for pili pulp oil isolation from the existing methods employed by pili processors. It likewise involved testing of the new protocol to determine its efficacy in isolating oil from pili pulp of Bicol varieties.

Existing Pulp Oil Isolation Methods

There were three methods noted for pili pulp oil isolation used by processors. These are enzymatic extraction, the DOST method, and the traditional method employed by local oil extractors.

1. Traditional extraction is a method employed by farmers/ pili pulp extractor in the Province of Sorsogon, patterned from the coconut oil extraction method. It involves pulping, pressing, and extracting, then cooking. The pulp was first softened by a local process called "paglanta"; extracting includes mixing, macerating, pressing, and filtering; cooking is heating the extract in a 'carahay" or big sauce pan, until the water components have entirely evaporated and the suspended solid particles have settled as residue. The oil is then transferred to the storage container.

2. DOST Method is composed of simple cooking and filtration tools that process oil extracts from freshly harvested pili nuts using minimal heat (Calleja, 2009). It includes pulping, extraction, filtration, and evaporation to separate the oil from the water. This method has been introduced to pili pulp oil entrepreneurs in Sorsogon by DOST Provincial Office in Sorsogon province. The technique is found friendlier to local processors not only because of its simplicity but also because the process is synthetic chemical-free.

3. Enzymatic extraction involves three operations: pre-treatment, extraction or oil release, and oil recovery (Pham and Pham, 2008). The process entails aqueous extraction involving mechanical and enzymatic degradation of the cell walls. The enzymatic process uses water as solvent and cell wall degrading enzymes to facilitate an effective and mild fractionation of oil, protein, and hulls. The oil found inside plant cells is linked with proteins and a wide range of carbohydrates like starch, cellulose, hemicellulose, and pectin (Pham and Dumanadan, 2015). The inner cell surrounded by a thick wall must be opened so the protein and oil can be released. Thus, when opened by enzymatic degradation, down -stream processing makes fractionation of the components possible to a degree, which is better than the conventional technique like pressing.

The three existing methods have features that can serve as the basis of the new protocol. The practical and feasible features were adopted, and some are considered in coming up with an improved feature to develop a relevant, community-based, and more user-friendly option for the pili pulp oil isolation process.

Knowledge and Innovative Technology Generated from Findings

Analysis of the existing methods employed in pulp oil isolation revealed three general processes: pulping, extraction, and oil isolation. There were, however, specific procedures employed that differed from the existing methods. This study found that maximizing the secretion of oil embedded in the pulp and fractionation of extract components are necessary for the process. Significant knowledge noted in the existing methods employed in pili pulp oil extraction is given in Table 1.

It can be noted from Table 1 that there are procedures employed in the existing methods in pili pulp oil isolation that can be improved to come up with an alternative or innovative method of pili pulp oil extraction. The features of the three existing protocols served as the basis of the new process.

Table 1. Knowledge noted, its implications, and alternative/innovative technology generated.

Procedures / Processes in Existing Methods	Purpose	Implication/s	Alternative/ Innovative Technology Generated
"Paglanta" or pulp softening process	To soften the pulp.	The process is not well defined as to what is the appropriate water temperature to use. There is a need to stabilize the pulping temperature to be employed.	Water temperature ranging from 50°C to 60°C may be employed in the pulping process.
Mechanical pulping macerating and pressing to get the pulp extract	To get the extract containing oil from the pulp.	To enhance the oozing of oil from the network of fibers in the pulp. May be facilitated by using/developing a machine that can do the processes together in one operation.	The use of blender/ similar machine may be introduced in the maceration process. It will facilitate the process and will make it clean and standardized.
Cooking/ Evaporation	To eliminate and separate the water impurity in the extract from the oil components.	Applying heat facilitates the evaporation of water and settling of suspended impurities as residue. Time consumed in carrying out the process may be shortened if the water will be separated by employing other means of separation.	Decantation, flotation and scooping by hand using appropriate scoop may be employed first before cooking/ evaporation. Fractionation of the mixture will save time, resources, and energy that will be utilized in the process.
Use of enzymes in Enzymatic Extraction	To degrade the cell walls in the pulp's fibers so that the oil embedded in it will be released and quickly oozed out of its network.	The procedure is intended to maximize oil isolation. If an alternative method of degrading the pulp's fibers be used to substitute the process, the use of enzyme and synthetic chemicals may be eliminated.	Natural fermentation of the extract may be introduced in the process of oil isolation to degrade the fibers.

In the traditional method the process of softening the pulp "paglanta" was done by approximating the temperature. However, it was noted that pili pulp, when soaked in water of higher temperature than what is necessary, would harden rather than soften, thus the need to investigate the appropriate temperature range that can promote softening of the pulp. Trials were made to determine a standard temperature appropriate to be employed.

The mechanical process of oil extraction needs to be mechanized for it to be facilitated. The use of existing appropriate machines like blender was introduced in the process and findings revealed that doing so really facilitated the maceration and it likewise standardized the procedure and make it more sanitary. It also implied that an all-in-one machine that can do not only maceration but also pressing and filtering may be designed/fabricated for more efficient mechanization of the process.

Innovation in cooking/evaporating the crude extract was introduced by employing fermentation, decantation and scooping or separation using a separatory funnel. The innovative procedure fractionates the crude extract, thereby reducing the volume of water and other impurities thus saving time, resources, and energy.

In the existing enzymatic extraction, the enzyme was used as a tool to degrade the network of fibers that holds the oil in the pulp. The decomposition process by fermentation is the innovative process introduced to facilitate and maximize the secretion of oil.

Figure 1 presented the RPOIM, a method of pili pulp oil isolation. It can be noted from the figure that the alternative protocol consists of three major processes such as extraction and fractionation, oil isolation, and oil recovery.

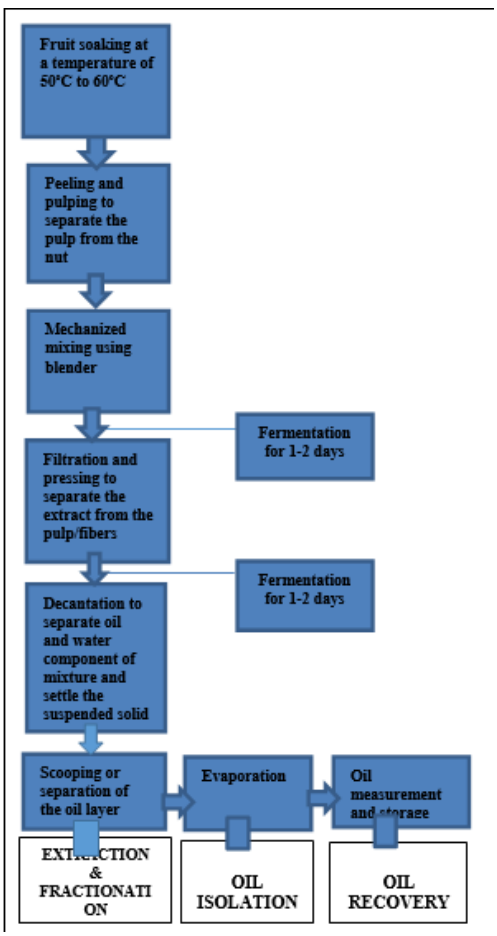

Fig. 1: Innovative/Alternative Pili Pulp Oil Isolation Method (IM-Ramirez Pulp Oil Isolation Method).

During the extraction and fractionation process, several steps were involved. Each of the steps was deduced from the analysis of the description matrix for the existing methods. There were 6 steps involved:

1. Fruit soaking at a temperature of 50°C to 60°C is intended to soften the pulp. Softening the pulp allowed easy extraction of the oil-containing liquid component which is naturally present in it.
2. Peeling and pulping to separate the pulp from the nut is done to obtain a material that can be subjected to an easy extraction process. The nut containing the kernel is hard and woody and must be separated and used for other purposes.
3. Mechanized mixing using a blender is for the degradation of the fibers in the pulp. It is in the network of fibers that oil content is embedded, thus must be destroyed for the oil to ooze out easily and maximize the oil recovery. Fermentation for 1-2 days is an added process in this part to enhance network degradation.
4. Filtration and pressing to separate the extract from the pulp/fibers is the process that resulted in the separation of the oil-containing liquid component. That component must be separated from the pulp for easy separation of the oil. Decomposition of the suspended solids left in the liquid mixture will be enhanced by the added process of settling it for another 1-2 days and to decay.
5. Decantation to separates oil and water component of the mixture and settled the suspended solid. This process will promote the layering of the oil-containing liquid.
6. Scooping and separation of the oil layer is the final process that yielded the oil but still with water impurities and suspended particulates.

Oil isolation is done by employing the process of evaporation intended to

purify the oil-containing layer obtained after scooping. Evaporation is the application of heat that allowed the phase change of the water from liquid to gas thus obtaining the pure pili pulp oil.

Oil recovery is the quantification and storage of the isolated oil after evaporating the water impurities.

Testing of the Alternative Method in Pili Pulp Oil Isolation

Testing the efficacy of the protocol for its intended purpose is one important aspect of establishing an alternative protocol to be recommended as an option to existing and commonly employed processes. RPOIM must be subjected to validation and testing to establish its potential for efficient and practical utilization.

Testing of the developed protocol was done using fruits of different varieties of pili from the Department of Agriculture (DA) Regional Office V such as Goa , Lanuza, Penafrancia, Magnaye, Laysa, and Sorsogon varieties. It was requested from DA to ensure authenticity for easy identification and labeling. Samples are shown in Figure 2.

It can be noted that generally, the color of pili fruit is black with purple characteristics. Some fruits are elongated and wide, while others are elongated but small. Some are rounded and short. The variety which was found bigger than the rest was Penafrancia and Goa followed by Magnaye and Laysa, Sorsogon and ever bearing varieties were noted to be smaller. Statistical test of significance on the average fruit mass however indicated that there are no significant differences in the size of the pili fruit samples used at .05 level of significance. It implied that visual

Fig. 2. Samples Used in Testing the Alternative Protocol.

attributes of size are not standard since these may be affected by the cultural management employed in pili production. Data was only based on the samples at hand.

Pulp of the samples were processed for oil isolation using the alternative protocol, the RPOIM. Result is consistent with the known fact that oil is present in pili pulp as indicated by the successful isolation of oil from majority of the samples. Oil yield is given in Table 2.

Data in Table 2 revealed differences in the oil yield of the samples but the results manifested applicability of the alternative method in isolating oil from pili pulp. Specifically, data indicated that Goa variety has the highest yield of 35.6% followed by Laysa with 25.2%. The rest of the samples showed lower percent yield. One significant finding about oil yield of pili pulp is its connection with the nature of the pulp.

Findings showed that oil yield is higher for varieties with fibrous pulp and thus a potential source of oil.

Those with starchy pulp may be considered for other uses.

Applicability of Knowledge and Technology Generated from Findings

The alternative method in pili pulp oil isolation introduced by the study may be used by pili processors who are producing bulk of pili pulp in the processing of fruits to obtain the kernels. It may be used by pili processing households who were not so inclined in adopting highly technical methods of oil isolation such as the enzymatic extraction. It is efficient and cost effective because it uses materials and tools available in the community thus may be regarded as a community-based method of pili pulp oil isolation.

Laysa and goa varieties were noted as potential sources of pili pulp oil and lanuza was likewise found to contain desirable quantity of pulp oil. Nature of the pulp is one good indicator of the oil yield because findings indicated that varieties with fibrous pulp yielded greater volume of oil compared to those with starchy pulp. Entrepreneurs focusing on pili pulp oil business may be advised to plant Laysa, Magnaye and Orbase varieties in their farms.

CONCLUSION

There are three existing pili pulp oil isolation methods employed by pili pulp oil extractors in the region such as traditional method, DOST recommended method and the enzymatic extraction. RPOIM was developed based on the features and noted deficiencies of the existing protocol. It is characterized by three generic processes such as extraction and fractionation, oil isolation and oil recovery. Fermentation of pulp for 1-2 days after mechanized mixing and after extraction is a distinct feature of the alternative method.

Testing of the new technology manifested applicability of the recommended option in pili pulp oil isolation as it was effectively used in determining percent oil yield of samples. It can be used by pili processors who are producing bulk of pili pulp in the processing of fruits to obtain the kernels. It may be used by pili processing households who are not so inclined in adopting highly technical methods of oil isolation such as the enzymatic extraction.

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ACID-BASE INDICATOR PROPERTIES OF GUMAMELA PETAL VARIETIES

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Abstract — Exhaustive survey on the utilization of Philippine resources revealed urgent need for continued effort on the chemical study of the country's flora for commercial, industrial and educational purposes. Gumamela is one of the plants that have to be developed for its many uses. Isolation of acid-base indicator from plant is not new in chemistry. Several hundreds of indicators have been investigated and the list continues to grow. This study investigated on the acid-base indicator properties of gumamela petal varieties.

Three gumamela petal varieties namely: *Hibiscus rosa-sinensis* var. 'Gelia Castillo', *Hibiscus rosa-sinensis* var. 'Petite Peach' and *Malvaviscus arboreus* var. 'Penduliflorus' were used. Testing includes determination of the phytochemical components of the extract and determining indicator properties in acidic and basic solutions.

Results showed different color changes in acid and base solution for all the samples. Supported by phytochemical analysis, the study concluded that the gumamela varieties tested possessed natural acid-base indicator properties with *H. rosa-sinensis* var. 'Gelia Castillo' having the highest potential. A standard color matrix for each gumamela variety was developed to serve as guide in interpreting its response to acid and base solutions. The color matrix will serve as standard in determining pH of solutions.

Keywords — **Acid-base, natural indicator, *H. rosa sinensis* L., acid-base color matrix, Malvaceae.**

INTRODUCTION

Gumamela or *H. rosa-sinensis L*. is a member of the Malvaceae family under the division Magnoliophyta. The plant is erect, highly branched, glabrous tree (Magdalita et al, 2019) with a height ranging from 90 cm to 6 m. The leaves are green, deltoid to ovate, measuring 7-12 cm and are continuously produced all year round.

Gumamela flowers are complete and perfect having the stalks holding the stamen and the pistil fused in one column emerging in the middle of flower (Ellis, 2017). It is commonly used for adorning home facades and gardens because of its beautiful color hues and color combinations, thus referred to as “Queen of Filipino Garden”.

More than its ornamental value, gumamela is also used as a herbal medicine to treat different ailments such as epilepsy, leprosy and diabetes (Kumar and Singh, 2012). It has also found its place as an important flower in education especially in the field of Science because of its viability as natural dyes, food flavor and coloring pigments. It has also find its way in the classroom as teachers are using it as a local indicator in the lesson for acid and bases.

About 300 species of *Hibiscus* plants are found worldwide. *Hibiscus* is regarded to be one of the most widely cultivated flowering plants in the tropics with short-lived but continuing blooms in brilliant hues of red, orange, purple, white and yellow since the original red-flower plant has undergone tremendous development. It is easily propagated and not particularly exacting in its growing requirements. It is connoisseur flower that gained countless fans like other flowers such as dahlias and rose (Magdalita, 2012). Some species of this plant are grown with double or semi-double petals for improving its aesthetic values. Most of its species represent some nations worldwide.

These flowering plants do not just range from its aesthetic value. It also serves a good source for medications to cure sickness, inflammation, and infections. The flowers are considered as astringent and yield polyphenols, flavonoids, anthocyanins and hisbiscetin. Studies also show that these flowers contain polysaccharides which promote wound healing and are immune modulating. The roots on the other hand, contain mucilage that is soothing on pethe mucous membranes as digestive and respiratory tracts (Stuart, 2016). Natural dyes is also an eco-friendly acid-base indicator (Pathan and Faroogui, 2011). *Hibiscus* plants are abundantly grown and cultivated in the native old world of many countries. Since it is most suitable for humid and tropical climates, great supplies can be found to tropical and subtropical countries.

Belonging to the family Malvaceae, gumamela (*H. rosa-sinensis*) is a common garden plant in the Philippines that produces large flowers, which measure about 4 inches in diameter. The flowers come in white, yellow, pink, red and thousands of color combinations, except true blue or black. Some smaller gumamela varieties have blossoms that are 2 inches in diameter and the flowers of other bigger varieties are 10 to 12 inches in diameter. The flowers come in single or double layers, depending on the variety.

Chemists perceived gumamela flowers as a complete chemical entity which is composed of different chemical compounds. Years of research in this plant species, great minds discovered that the fragrance in flowers is due to terpenes and terpenoids. The color is due to flavones, flavanols, anthocyanins and other phytochemicals and its nectar is a mixture of sweet carbohydrates. It

has many applications where flowers can be used. One such application is the use of flower extract in acidimetry and alkalimetry as an indicator (Okoduwa et al., 2015).

The isolation of acid-base indicator from plant is not new in the field of chemistry. Several hundreds of these indicators have been investigated and the list continues to grow. Gumamela petal, as a possible source of acid-base indicator offers the advantage because it is generally considered a waste material and indigenous in the Philippines. Natural indicators must be developed for healthy environment and to find alternative materials that are available in the locality especially to far – flung barangays, thus this study.

The study investigated on the acid-base indicator potential of *H. rosa-sinensis* var. 'Gelia Castillo' var, *H. rosa-sinensis* var. 'Petite Peach' and *Malvaviscus arboreus* var. 'Penduliflorus'. It included the conduct of phytochemical analysis to determine the chemical nature of the materials. The responses of the extracts to acid and base samples were likewise noted from where a color matrix was developed as bases of the acid-base indicator properties. It however, does not consider other gumamela varieties which are not commonly available in the locale.

MATERIALS AND METHODS

The study involved three major phases such as: a) establishing phytochemical components of the samples, b) testing for the natural acid-base indicator properties, c) analysis of data and developing a color matrix for gumamela varieties. It was however preceeded by selecting a potential plant material that showed the characteristics of a potential natural acid-base indicator.

Collection and Description of the Raw Materials

Three varieties of gumamela flower were used in this research. These varieties are those that are common, dominant and can easily be found in the community. *H. rosa-sinensis var. 'Gelia Castillo'* (Fig. 1), *H. rosa-sinensis var. 'Petite Peach'* (Fig. 2) and *Malvaviscus arboreus var. 'Penduliflorus'* (Fig. 3) obtained from the Camarines Sur, Bicol Region, Philippines were used in the study.
H. rosa-sinensis '*Gelia Castillo*' has

Fig. 1. *H. rosa-sinensis* var. '*Gelia Castillo*'.

Fig. 2. *H. rosa-sinensis var. 'Petite Peach*'.

Fig. 3. *Malvaviscus arboreus var.*

gold petals with deep maroon eye zone and orange halo. Flowers are large to extra-large, single, and with overlapped blooms. Plants have very prolific blooming habit, tall, upright bushy, and are well-branched. It is part of the UPLB Millenium H. Hybrids Series.

H. rosa-sinensis '*Petite Peach*' has peach flowers with a red eye and white halo. It has lanceolate and serrate leaf margins. It is a shrubby variety that is floriferous and vigorous. The variety produces small to medium sized flowers that are one day-old with 5 single type petals.

Malvaviscus arboreus var. *'Penduliflorus*' is a perennial, tall evergreen shrub that produces tubular, pendulous. Solitary flowers that are 6.4 cm long. It has multiple anthers. The flowers are drooping that never open. Leaves are dark green with alternate phyllotaxy, lancearate to ovate with a pointed tip.

Reasonable quantity of *H. rosa-sinensis* var. '*Gelia Castillo*', *H. rosa-sinensis* var. '*Petite Peach*' and *Malvaviscus arboreus* var. '*Penduliflorus*' were collected and the petals were thoroughly air-dried for three days. The 20g dried samples for each gumamela variety was used for the phytochemical analysis.

Establishing Phytochemical Components of the Samples

The highly colored pigments obtained from plants are found to exhibit color changes with variation of pH. The chemical substances which possess an apparent change in color of analyte and titrant reacting mixture very close to the point in the ongoing titration is known as indicator. Typically, the presence of flavonoids and alkaloids may result to sharp color changes (Hossain et al., 2013) which had occurred at end point of titrations. Thus, phytochemical analysis, a test to know the presence of flavonoid and alkaloid in the samples was employed.

***Collection and preparation of materials*.** The samples of *H. rosa-sinensis* var. 'Gelia Castillo', *H. rosa-sinen*sis var. 'Petite Peach' and *Malvaviscus arboreus* var. 'Penduliflorus' were collected in the province of Camarines Sur Philippines.

***Sample preparation*.** Appropriate quantity of *H. rosa-sinensis* var. 'Gelia Castillo' (sample 1), *H. rosa-sinensis* var. '*Petite Peach*' (sample 2) and *Malvaviscus arboreus* var. '*Penduliflorus*' (sample 3) were prepared and the petals were thoroughly air-dried for three days. The 20g dried samples was used for the phytochemical analysis.

***Phytochemical Analysis*.** The air-dried 20 g each of *H. rosa-sinensis* var. '*Gelia Castillo*', *H. rosa-sinensis* var. '*Petite Peach*' and *Malvaviscus arboreus* var. 'Penduliflorus samples were subjected to preliminary phytochemical screening to identify the chemical constituents at the Department of Science and Technology (DOST) Laboratory, Taguig, Philippines. Test for the presence of anthocyanins, specifically alkaloids, flavonoids, phenols and

tannin using appropriate and standard method of phytochemical analysis.

Testing for the Natural Acid- Base Indicator Property

Laboratory testing to determine the potentials of extracts from the gumamela test varieties was employed. It was done through the following processes:

Plant Collection. Fresh samples of *H. rosa-sinensis* var. 'Gelia Castillo', *H. rosa-sinensis* var. 'Petite Peach' and *Malvaviscus arboreus* var. 'Penduliflorus' were collected in the province of Camarines Sur,Philippines.

Sample Preparation. Fifteen pieces of petals each of fresh *H. rosa-sinensis var. 'Gelia Castillo', H. rosa-sinensis var. 'Petite Peach'* and *Malvaviscus arboreus var. 'Penduliflorus'* were pounded using mortar and pestle and its juice was extracted using 15 ml of distilled water as the solvent. Extracts were labeled as Sample A for variety no. 1 (*H. rosa-sinensis var. 'Gelia Castillo'*), Sample B for variety no. 2 (*H. rosa-sinensis var. 'Petite Peach'*) and Sample C for variety no. 3 (*Malvaviscus arboreus var. 'Penduliflorus'*). The extracts were filtered and reserved for the actual laboratory test.

Experimental Design and Treatments. To determine the response of gumamela extracts to the acid-base solutions, a complete block design was employed. Standard acid and base solutions was prepared and used in the testing according to the experimental lay-out in table 1.

Table 1. The Experimental Lay-out.

Treatment 1 H. rosa-sinensis var. 'Gelia Castillo'			**Treatment 2** H. rosa-sinensis var. 'Petite Peach'			**Treatment 3** Malvaviscus arboreus var. 'Penduliflorus'		
pH 2	pH 7	pH 10	pH 2	pH 7	pH 10	pH 2	pH 7	pH 10
R1	R1	R1	R1	R1	R1	R1	R1	R1
R2	R2	R2	R2	R2	R2	R2	R2	R2
R3	R3	R3	R3	R3	R3	R3	R3	R3

Legend: R1-replicate 1; R2- replicate 23 R3- replicate

Three replicates each for every experimental treatment were tested Treatment 1 was *H. rosa-sinensis var. 'Gelia Castillo'reagents*; treatment 2 was *H.* rosa-sinensis var. 'Petite Peach' and treatment 3 was *Malvaviscus arboreus* var. 'Penduliflorus'. Each sample was tested with standard acid and base solutions of pH 2, 7 and 10. The main intent was to test the extracts with all the pH from pH 1 to pH 14 but the constraint of not having buffer for all the pH in the laboratory limited the conduct of the testing. Hydrochloric acid was the acid solution used and Sodium Hydroxide was the basic solution. The chemicals were particularly chosen because aside from being the common acid and base, it is also the strongest acid and base respectively.

Testing the indicator properties of the extracts. The filtrate (extract) of each flower was tested with acid and base solutions and the results were recorded. Three drops of the extract were added into each of the sample solution and the response was determined by observing any color change that will take place.

Analysis of Data and Development of Color Matrix

Observation about the color response of the various treatments tested to the standard acid and base solutions was used as basis of developing the standard color matrix for each of the gumamela species that can be used as a guide in using the natural indicator from gumamela plant species tested. UV Spectrophotometer was used to determine the wavelength of the color range to get the accurate color for each of the sample response.

RESULTS AND DISCUSSION

Acid-base indicator property of gumamela varieties were discussed on the following results. It was focused on a) phytochemical components of the extract; b) response of the gumamela extracts to standard acid and base solutions; c) color matrix that was developed from the color responses of the experimental extracts.

Phytochemical Components of the Samples

Phytochemicals are naturally occurring plant chemicals. They provide plants with color, odor, and flavor. Brightly colored plants are often sources of phytochemicals. Thousands of phytochemicals have been identified and scientists begun to investigate their promise. Some of the phytochemicals are carotenoids, flavonoids such as anthocyanins, indoles and glucosinolates, inositol, isoflavones, polyphenols and terpenes.

Table 2 presents the results of phytochemical analysis to the samples. Data reflected positive results about the phytochemical contents of the sample extracts tested. It can be gleaned from table 2 that sample extract 1 contains seven phytochemicals such as sterols, triterpenes, flavonoids, alkaloids, saponins, glycosides and tanins. Triterpenes, flavonoids and tannins are the most abundant phytochemicals.

Table 2. Results of Phytochemical Test for Gumamela Varieties Tested.

Phytochemical	Samples		
	S1	S2	S3
Sterols	++	+	++
Triterpenes	+++	+++	+++
Flavonoids	+++	++	++
Alkaloids	+	+	(-)
Saponins	++	++	+
Glycosides	++	++	++
Tannins	+++	+++	+

Legend: S1- *rosa-sinensis var Gelia Castillo*
S2-rosa-sinensis var Petite Peach
S3-
Note: (+) traces; (++) moderate
(+++) abundant (-) absence of constituent

Sample extract 2 (*H. rosa-sinensis* var.'Petite Peach') likewise contains all the phytochemicals found in sample 1 but differ in the relative quantity. It was noted from the results that tripertenes and tannins are the phytochemical present in abundant quantity in the sample.

It can be noted further in table 2, that sample extract 3 (Malvaviscus arboreus var. 'Penduliflorus') contains phytochemicals such as sterols, triterpenes, flavonoids, saponins, glycosides and tannins. It was found not to contain alkaloids. It was further indicated in the results that triterpenes is the most abundant phytochemical in the sample.

Findings revealed that the three species tested contain the same phytochemical component except *Malvaviscus arboreus* var. 'Penduliflorus' which was found not to contain alkaloids.

Looking closely in the results, it can be gleaned that in terms of sterols, sample1 has a moderate content compared to trace content for both samples 2 and 3. All of the three varieties have abundant amounts of triterpenes.

In terms of flavonoids, sample 1 has an abundant content compared to moderate content for both samples 2 and 3. Samples 1 and 2 contain trace amounts of alkaloids while sample 3 does not contain it. Saponin is moderately observed in sample 1 and sample 2 but only trace amounts is observed in sample 3.

Glycosides were likewise found to be moderately present in all the three varieties. Tannin is abundant in samples 1 and 2 but only trace amount in sample 3.

It can be inferred from the results that gumamela varieties are of different chemical makeup. There may be similarities in the phytochemical components but generally it differ in the detectable amounts. It logically mean that there is a variation in the chemical constituents of petal extracts even if they were in the same family thus imply variations in the response to acids and bases. It may further imply that variations in the color matrix for responses to different pH of the varieties is possible.

Results of the study supported the findings of Magalong and Lopez (2007) on the determination of phytochemical content and antimicrobial properties of floral extracts from 14 cultivars of gumamela (*H. rosa sinensis*). The study revealed that all the floral extracts from the different gumamela cultivars were found to contain alkaloids, cardenolides and bufadienolides, tannins, protein and carbohydrates.

Results likewise conformed with the preliminary result of the phytochemical analyses, nutrient value and economic importance of *H. sabdariffa*, a gumamela species in Nigeria as reported by Okereke et. al. (2015). It showed that there are some plant chemicals present in the extract such as alkaloids, tannins, saponnins, glycosides, phenols and flavonoids.

Potentials as Acid-Base Indicator

Relating results of phytochemical analysis of the samples to its potential as natural acid and base indicator, it can be inferred that all the three samples can be a natural acid and base indicator because all of them contains flavonoids.

Flavonoids are a ubiquitous group of naturally occurring polyphenolic compounds characterized by flavan nucleus and represent one of the most prevalent classes of compounds in fruits, vegetables and plant-derived beverages. Anthocyanins are group of flavonoid compounds. The results obtained in all the types of acid-base titrations lead us to conclude that the presence of flavonoids made possible the sharp color changes that occurred at end point of titrations (Burungale and Mali, 2014).

Foregoing results of phytochemical analysis for all the 3 samples revealed presence of flavonoids thereby indicating potential of samples as acid-base indicator. The result conformed with the study of Magalong and Lopez (2007) and Okereke et. al.(2015); that flavonoids were present in H*ibiscus* varieties.

Responses of Gumamela Extracts to Acid and Base Solutions

Indicators are chemical substances added in small quantity to a solution to determine the acidity or alkalinity of the solutions (Garba and Abubakar, 2012). Indicators are weak organic acids or bases that exist in more than one structural form (tautomers) of which at least one form is coloured. Natural acid - base indicator helps determine the pH value of a given substance. Common natural indicators are pigment, known as anthocyanin, which reacts very visibly to changes in pH.

Table 3. Color Change of Gumamela.

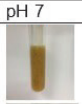
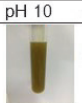
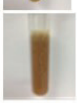
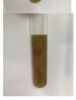
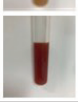
T	pH 2	color	pH 7	color	pH 10	color
1		pink		Dark yellow		green
2		red		Light pink		Dark green
3		Dark red		Red violet		violet

Table 3 presented the color responses of the sample extracts to the changes in pH with its corresponding changes in color. Three varieties of gumamela

were investigated at pH 2, 7 and 10 and the color change was observed along with the changing pH.

Results of titration indicated that the three extracts tested have varying response to pH changes as indicated by the changes in color observed. Findings imply that color matrix vary among three varieties and thus have different responses and potentials as natural indicator.

Results of this study revealed that *H. rosa-sinensis* var. '*Gelia Castillo*', *H. rosa-sinensis* var. 'Petite Peach' usually turns to red or pinkish red in acidic (pH 2) titration while green to dark green in basic (pH 10) titration respectively. The extract of *Malvaviscus arboreus* var. '*Penduliflorus*' was observed to have a somewhat different color response among the varieties tested because the color changes from dark red to purple as it was titrated with acidic and basic solutions (pH 2 to pH 10). This study found out that the color changes of the extracts change as the pH changes for all samples tested thereby indicating its potentials as a natural acid base indicator.

Verification with UV Spectrophotometer

These results were supported by the data from UV Spectrophotometer that was used to determine the wavelength of the color range to get the accurate color for each titration. The figures below presented the absorbance and transmittance value of the extracts.

The Maximum absorption (λ_{max}) of the initial color of the petal extract of *H. rosa-sinensis* var. '*Gelia Castillo*'. is 400 nm and a transmittance of 450 which corresponds to the color yellow (Figure 4).

The Maximum absorption (λ_{max}) of the color of the petal extract of H. rosa-sinensis var. '*Gelia Castillo*' in basic titration (Figure 5). It has an

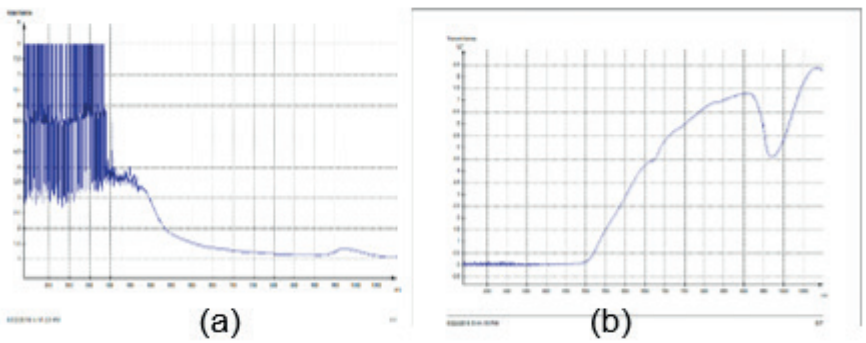


Fig. 4. Absorbance (a) and transmittance (b) of sample 1 before titration

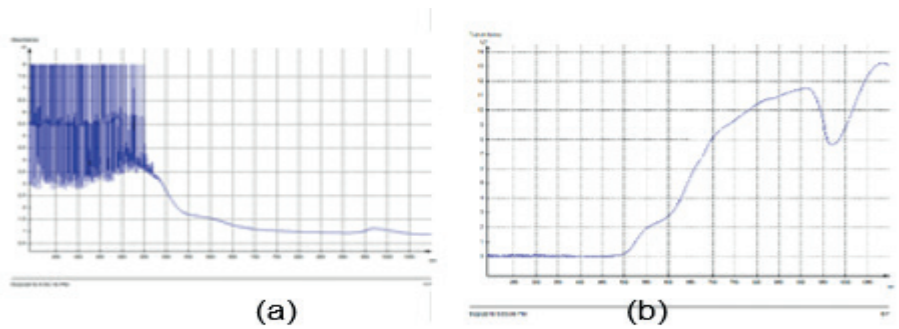


Fig. 5. Absorbance (a) and transmittance (b) of Sample 1 in basic solution.

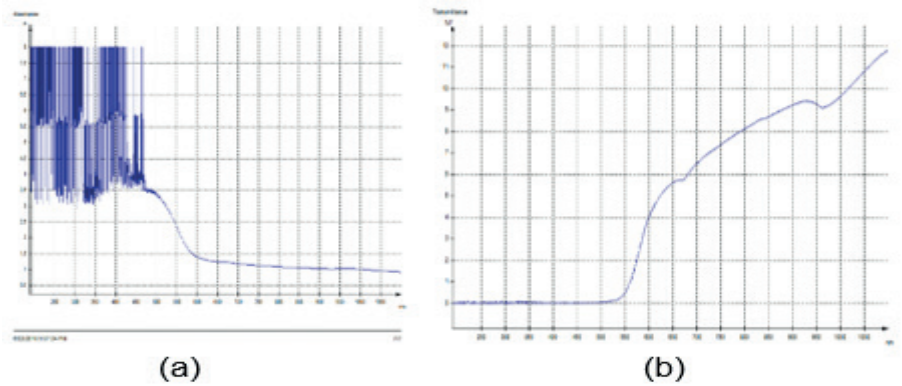


Fig. 6. Absorbance (a) and transmittance (b) of sample 1 in acidic solution.

absorbance of 500 nm and a transmittance of 450 nm which corresponds to the color green.

The Maximum absorption (λ_{max}) of the color of petal extract of H. rosa-sinensis var. 'Gelia Castillo' in acidic titration (Fig. 6a). It has an absorbance of 500 nm and a transmittance (Fig. 6b) of 490 nm which corresponds to the color red.

The Maximum absorption (λ_{max}) of the initial color of the petal extract of *H. rosa-sinensis* var. '*Petite Peach*' (Fig. 7). It has an absorbance of 400 nm and a transmittance of 430 nm which corresponds to the color pink.

The maximum absorption (λ_{max}) of the color of the petal extract of *H. rosa-sinensis* var. '*Petite Peach*' in basic

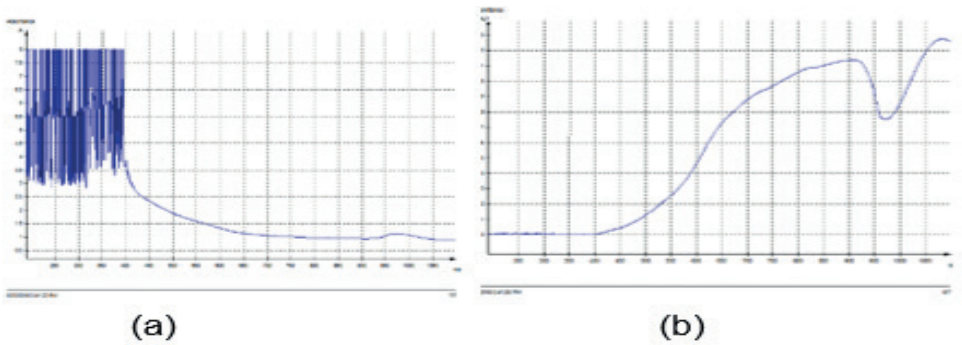


Fig. 7. Absorbance (a) and transmittance (b) of sample 2 before titration.

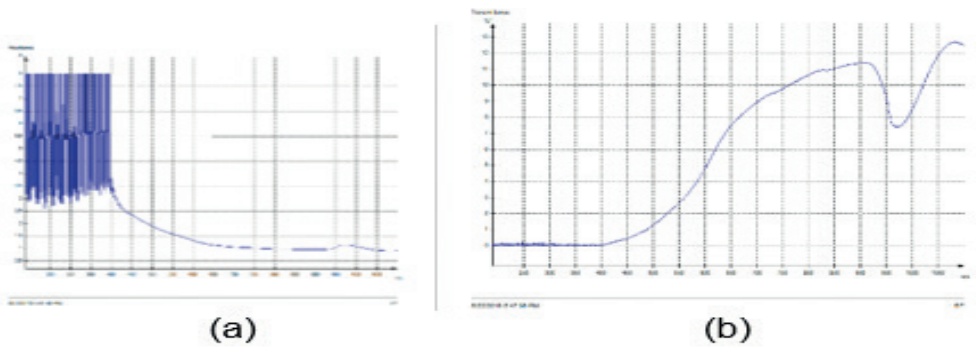


Fig. 8. Absorbance (a) and transmittance (b) of sample 2 in basic solution.

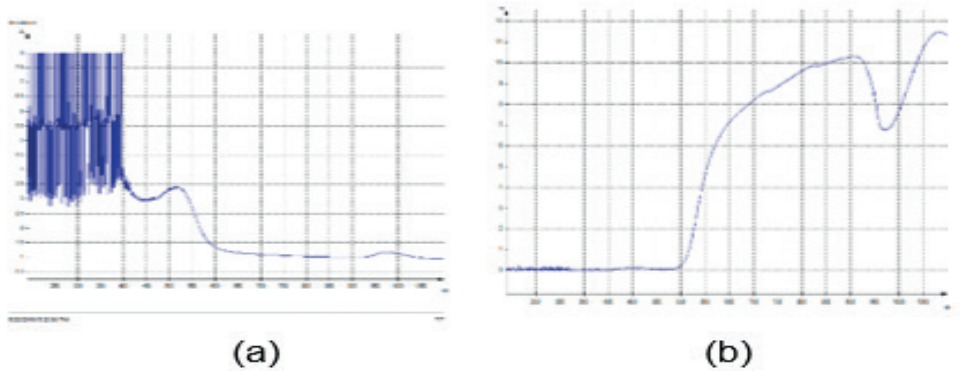


Fig. 9. Absorbance (a) and transmittance (b) of sample 2 in acidic solution.

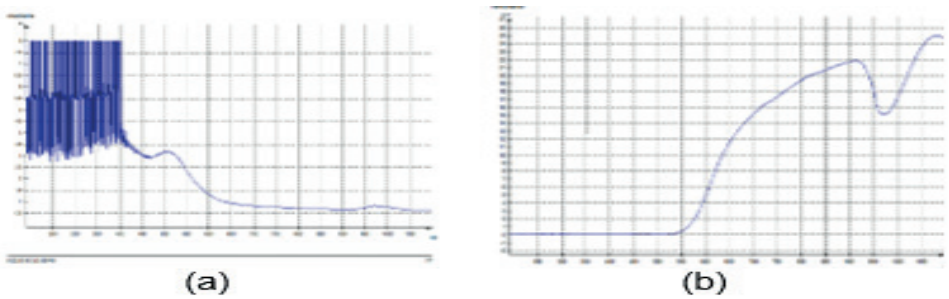


Fig. 10. Absorbance (a) and transmittance (b) of sample 3 before titration.

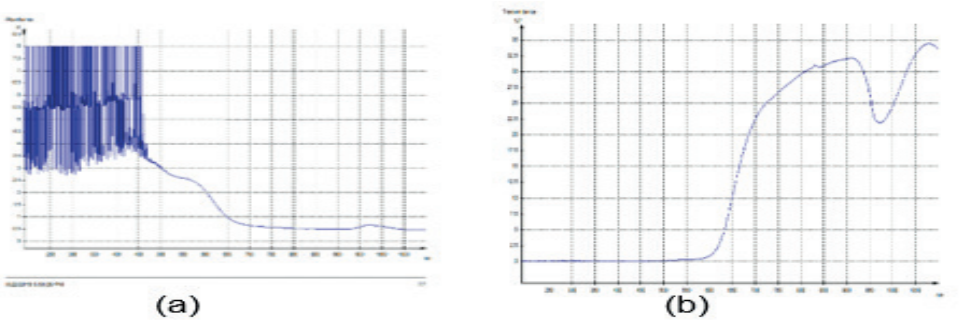


Fig. 11. Absorbance (a) and transmittance (b) of sample 3 in basic solution.

titration. Its absorbance is 400 nm and a transmittance of 400 nm which corresponds to the color green.

The maximum absorption (λ_{max}) of the color of the petal extract of *H. rosa-sinensis* var. '*Petite Peach*' in acidic titration 520 nm (Fig. 9) and a transmittance (Fig. 9b) of 500 nm which corresponds to the color red.

The Maximum absorption (λ_{max}) of the initial color of the petal extract of *Malvaviscus arboreus* var. '*Penduliflorus*' is 500 nm (Fig. 10) and a transmittance at approximately 500 nm which corresponds to the color red.

The maximum absorption (λ_{max}) of the color of the petal extract of

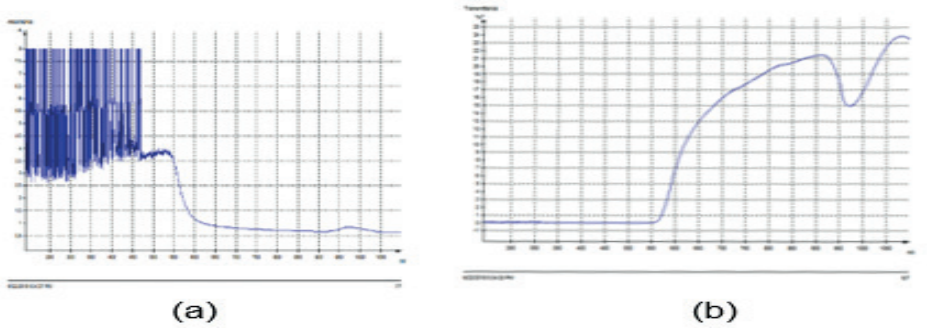


Fig. 12. Absorbance (a) and transmittance (b) of sample 3 in acidic solution.

Malvaviscus arboreus var. '*Penduliflorus*' in basic titration. It has an absorbance of 500 nm and a transmittance of 530 which corresponds to the color dark red to violet.

Noted in Fig. 12, that the maximum absorption (λ_{max}) of the color of the petal extract of *Malvaviscus arboreus* var. '*Penduliflorus*' in acidic titration is 550 nm and the transmittance is approximately 490 nm which corresponds to the color red.

Results of the study revealed that the color changes were in the visible region of the spectrum which is commonly from 200nm – 720nm. It was also observed that there was a shifting of color in each pH. In addition, the color changes observed with the gumamela extract has a good agreement with the prescribed colours of anthocyanin in the itemized reagents. These results indicate the presence of anthocyanin in the petals of the three gumamela varieties, and their color changes with different pH thus a potential natural acid-base indicator.

The absorbance vs. wavelength peak results of this study is similar to the work of Senathirajah (2017). In the acidic pH, such as pH<4, the absorbance maxima were observed at 520 nm, whereas a red-shift was attained with the pH>4. The peak maximum was recorded at 529.5 nm, 537.5 nm and with the pH 4, 5, and 6, respectively. The solutions at pH 8, 9, and 10 showed further red shift and the absorbance maxima was recorded at 560.0 nm, 549.5 nm and 568 nm. The solution at pH 11 and 12 show the peak maxima at around 580 nm, however, the intensity seems to decrease with the solution of pH 12. In particular, no peak maxima were observed in the visible region for the extract at pH 13 and 14, indicate that there was a lost in the conjugation of the anthocyanin chromophore. These results indicate that the H. extract act as a potential indicator over a range of pH.

Results of this study further implied that aqueous extraction does not alter the pH level of the extracts. Thus, when doing or using the natural extract indicator, distilled water must be used. The result also revealed that natural acid-base indicator shifted its color as it shifted its pH.

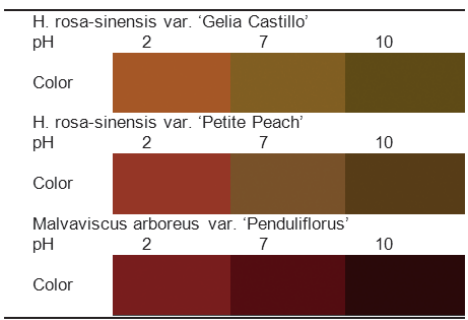
Color Matrix of the Plant Samples

Acid-base indicators are important because they help chemists get an estimate of the pH value of a given substance**.** These indicators can be used to classify substances as acids or bases, which are two important

classifications in the world of chemistry. Natural indicators relate information by demonstrating shifts in color to inform observers of whether materials are acidic or basic. The indicators, which are plants containing useful chemicals relative to pH levels however, do not provide a numeric value for acidity or basicity. Common natural indicators are litmus, red cabbage and *Hydrangea*. Anthocyanin in natural indicators reacts very visibly to changes in pH. There is a definite color matrix for a plant extract's response to acid and base solutions.

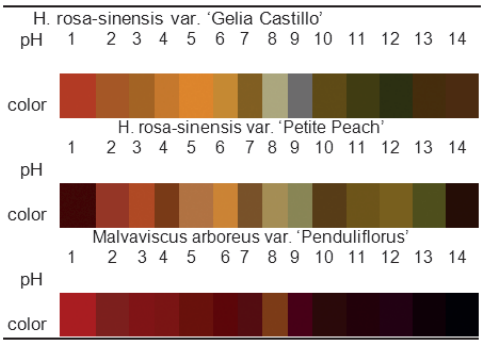
Responses of the various extracts tested to the acid and base solutions were used as basis in developing a standard color matrix for every gumamela species. Table 4 gives the color matrix developed from the responses of the gumamela extracts to standard acid and base solutions u sed in the laboratory procedures such as pH 2, 7 and 10 representing acid, neutral and base solutions. Observed color for the samples used in testing served as basis in coming up with a standard color matrix for all pH ranges.

Table 4. Color matrix developed from the responses of the 3 treatments tested to standard acid and base solutions.



The matrix will define the natural indicator's response to acid and base solutions at various pH, thus will serve as a guide in coming up with an approximate pH of a test solution. Interpolation on the observed color range for each of the test extract resulted to the Standard Color Matrix for each gumamela variety given in table 5.

Table 5. Color Matrix for *Hibiscus.*



The results supported the findings of Jain et al. (2012) that aqueous petal extract of flowers shows yellow for weak base and greenish in strong base while pink to red in weak acid and strong acid at end point (Jain et al. (2012). It also conformed with the result of the study of Gupta et al., (2012) where the color changed from pink to greenish yellow in th case of aqueous floral extract of H. rosa *sinensis* as natural indicator and the color changed from pink to green in the case of methanolic floral extract of *H. rosa sinensis* as natural indicator. Statistically also the use of natural indicator in acid base titration is proved, hence aqueous extract can be used with cent percent reliability and accuracy for acid base titration. Thus, the use of natural indicators in acid base titration is more beneficial because of their economy, easy to prepare, simplicity, easy availability, pollution free, inert and accurate results.

Potential Natural Acid-Base Indicator

The use of local materials has been the subject of cost oriented study by scientists over the years. Flower is one of such local (non-conventional) material that could be used in place of

standard indicator in science teaching (Garba et al , 2012).

In this study, the results of extracts color changes in acid and base solutions, showed a wide array of shifting. Since, the extracts produced color changes; it implied that they can be used to detect acidity or alkalinity of solutions. It also revealed the potentials of flower extracts as indicator and confirmed the assertion that; nearly all brightly colored flowers can be used as indicators. Thus, all of the three varieties were potential natural acid-base indicator however; the best variety that showed sharp color changes at end points due to the relatively wide shift in absorption and transmittance value which falls on visible region of spectrum that can be associated to its abundant content of flavonoids was the *H. rosa-sinensis* var. 'Gelia Castillo'.

The findings therefore, indicate an alternative way of equipping laboratory with practical instructional material (indicator) using plants that are around or within the environment.

CONCLUSION

The phytochemical components of the three gumamela varieties tested were sterols, triterpenes, flavonoids, alkaloids, saponins, glycosides and tannins. The findings also revealed that the three varieties contained the said constituents except for the alkaloids that is not found in *Malvaviscus arboreus* var. 'Penduliflorus'. Responses of the gumamela extracts were as follows: *H. rosa-sinensis* var. 'Gelia Castillo' extract exhibited a color change of red at pH 2 (acid), dark yellow at pH 7 (neutral), and green at pH 10 (base). *H. rosa-sinensis* var. 'Petite Peach' presented a color change of red at pH 2, pink at pH 7 and dark green at pH 10. *Malvaviscus arboreus* var. 'Penduliflorus' on the other hand showed a color change of dark red at pH 2, red violet at pH 7 and violet at pH 10.

Analysis of the noted response of the test extracts to acid base solution and interpolating the results yielded the standard color matrix for each gumamela variety. All of the three varieties were potential natural acid-base indicator however the best variety that showed sharp color changes at end points due to the relatively wide shift in absorption and transmittance value which falls on visible region of spectrum that can be associated to its abundant content of flavonoids was the *H. rosa-sinensis* var. 'Gelia Castillo'.

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MORPHOLOGICAL CHARACTERIZATION OF TARO (*Colocasia esculenta*) CULTIVARS IN THE BICOL REGION, PHILIPPINES

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Abstract — The study focused on the characterization of taro, scientifically known as *Colocasia esculenta* in the Bicol Region, Philippines. Taro samples were collected from different provinces in the Bicol region and planting materials for each sample were grown in a germplasm collection site. Characterization of cultivars was based on the established botanical morphometrics such as length, width and diameter for leaf, petiole and corm using an ordinary ruler and meter stick. Color variations of leaf margin, dorsal and ventral blade and veins, petiole and corm flesh and fiber were identified.

Results indicated 17 taro cultivars, 13 of which were identified with its local names as Princesa, Agatpaya, Balitaka, Sinamar, Tinahig, Quinsol, Negrito, Lipod/Ipod, Binting dalaga, Ito-ini, Inuruon, kolduroy, Duguan/ Dugong dalaga while four (4) cultivars were unknown to the farmers. Sample of cultivars were subjected to morphological characterization using color of leaf or blade, petiole and corm and sizes as major distinguishing characteristics.

Leaf color ranged from dark green on the dorsal to pale green on the ventral surfaces but varied in distribution of purple color. Petiole color varied from purple to green or a combination of both. Corm color is from white to yellow flesh with fibers from yellow to purple.

Keywords — **Taro cultivars, characterization of corm, leaf blade, petioles.**

INTRODUCTION

Taro, gabi, natong scientifically known as *Colocasia esculenta* is a crop that has been maintained by farmers for millennia (Rao et al, 2010). Taro genetic resources have remained largely under the control of local communities. The history of its adaptation, use and diversification can teach us many things about using and conserving genetic resources.

The plant, occupies a significant place in the agriculture of the Asia-Pacific Region. It is in this region, more than any other in the world, that the crop attains its greatest importance as a staple food (FAO, nd). It is particularly important for food security since many tropical areas often experience unfavorable environmental conditions (Beyene, 2013). Corms and leaves are usually consumed, taro supplies much-needed protein, vitamins and minerals, in addition to carbohydrate energy.

The socio-cultural importance of taro in the region is very high. The crop has evolved to be an integral part of the culture, and features prominently in festivals, social gift-giving and the discharge of social obligations. More recently, taro has become a source of income for individuals, and an earner of foreign exchange. Its role in rural development has therefore been increasing, especially with respect to the provision of employment and the alleviation of rural poverty.

The highest genetic diversity and number of private alleles were observed in Asian accessions, mainly from India (Chairi et al, 2016). There were also data from the Philippines but none has been specifically published from the Bicol Region. Having a database on the characteristics of existing cultivars in the locale may support future endeavors involving the important resource of the region. Detailed descriptions of accessions based on morpho-agronomical characters have tremendous impact on the conservation and genetic improvement of the crop (Beyene, 2013).

Demand for taro continuously increase due to export of taro related products to different parts of the world (Manner and Taylor, nd; Tridge, nd) and more and more farmers are indulged in planting it usually in rice fields or lowland areas. These farmers have rich experiences in growing taro but never been documented creating a vacuum with regards to the basic knowledge needed in taro research and development in the region.

Local farmers rely on indigenous knowledge and practices in their farming and production initiatives. One significant knowledge which was not yet explored is the identification and creation of database on the existing genetic resources of taro in the region. Identification of varieties that can be grown in specific geographical location and ecological ecosystems may contribute to the management of this crop for a bounty harvest. It will also serve as guide to farmers on the proper cultivar to propagate on certain situations and purposes.

Common names for taro cultivars noted from the farmers is one important indigenous knowledge because it can be a tool to facilitate easy identification and exchange of the planting materials. Characterization of the plant parts (leaf, petiole and corm) for each variety may serve as feeders in the determination its potential uses.

There were many economic benefits derived from taro. The leaves can be preserved or dried, and are an important food in times of scarcity. Petioles and stolons are also eaten fried or pickled. The inflorescence (a flowering stalk) is a delicacy in some food cultures of Asia and the Pacific. The corms and cormels are the most widely consumed plant part, the leaf blades, petioles, stolons, and inflorescences are also eaten,

depending on the cultivar and local food habits (Mathews et al 2017). Corms and leaves are also used for medicinal purposes. Having these parts characterized for each cultivar will introduce baseline data on the appropriate cultivar to be planted based on the purpose or intended utilization.

The undertaking focuses on the identification and characterization of taro (*C. esculenta*) cultivars in the Bicol Region. It determined the 1) available cultivars and characterized the color of the leaves, petiole and corm for each cultivar; and 2) established a germplasm collection of taro cultivars in the region.

MATERIALS AND METHODS

Description of the Locale

Research was conducted in the Bicol Region, Philippines wherein most of the research activities were done at the Central Bicol State University of Agriculture, San Jose, Pili, Camarines Sur (CBSUA). Bicol region comprises the southern part of Luzon, the largest island in the Philippine archipelago consist of six provinces, two of which were island provinces. The total land area is 5.9% of the total land area of the country. Around 69.3% of the total land area is alienable and disposable while the remaining 30.7% is public forest areas. The coordinates is 13° 30′ 0″ N, 123° 19′ 48″ E. The environment is very conducive for the production of C. *esculenta*, making it one of the commodities associated with the locale.

Cultivars Evaluated

Samples considered in the evaluation were those that are obtained after a regional collection of taro cultivars. Every cultivar noted to possess different characteristic/s from the other cultivars in the pre-evaluation were assumed a different sample variety. Taro seed pieces were collected from identified cultivars. The collected planting materials were grown in production site located at CBSUA compound and Pawili, Bula to produce enough samples for the morphological characterization. There were a total of 17 distinct cultivars subjected for characterization. Thirteen of which have local names derived from the farmers being interviewed while the rest are unknown.

Morphological Data Collection

Characterization was done 6 months after planting to ensure a well-developed mature plant parts. Botanist has been consulted for morphological parameters as basis of characterization such as color of the dorsal and ventral surfaces, vein, central portion and margin of the leaves, color of petiole and color of the corm flesh and skin. Leaf area, length of the stem and corm length and diameter were measured to establish the plant height as to small medium or tall variety. Data were recorded on individual plant basis.

Establishment of Germplasm Collection in a Field Genebank

Many important varieties of field, horticultural and forestry species are either difficult or impossible to conserve as seeds (Saad and Rao, 2001) because either no seeds are formed or if formed, the seeds are recalcitrant. One plant of such kind is taro, field genebanks (FGB) are commonly used for the conservation of taro genetic resources in the Pacific and other taro growing regions (Ebert and Wagainabete, 2018).

Planting materials of seventeen taro cultivars were collected, fifty seed pieces for each cultivar were planted in a designated site in the field genebank. One site of fifty plant per cultivar was maintained. Traditional farming system was employed in planting the seed pieces of taro with periodic weeding and cleaning of the plant where the old matured leaves were removed.

RESULTS AND DISCUSSION

Taro (*C. esculenta*) is an important member of the Araceae family and a staple food crop in many countries in the humid tropics and subtropics (Mathews, Lockhart and Ahmed, 2017). Before the start of the global trade and transport of agricultural commodities, taro was the world's most widely cultivated starch crop (Saad and Rao, 2001).

It is believed that taro originated in the tropics ranging from India to Indonesia (Mathews, 2004). This is supported by genetic diversity studies conducted by Chaïr et al (2006) which revealed that diversity was greater in accessions obtained from Asia compared to the Pacific, Africa, and the Americas. Within the Asian gene pool, India presented the highest numbers of alleles and private alleles.

In the Philippines particularly in the Bicol Region, taro is one important commodity because of its importance to the food security and regional economy. The region housed a number of taro cultivars grown by farmers in their farms that became their source of livelihood.

Taro Cultivars and its Characteristics

There are a number of taro cultivars noted in the Bicol Region. Results of this study revealed 17 different cultivars, 13 of which were locally named by the farmers while four were not identified.

Characteristics of the Blade

Blade or leaf is one important part of the taro plant. It has been a material used in many of the technologies or food products made from taro for commercial and economic purposes.

Shown in Table 1 are the taro cultivars and the morphological characteristics of its blade.

There were seventeen distinctly different cultivars of taro in the six provinces of the region. Those known and commonly identified by the farmers were ito ini (C2*), duguan* (C3), sinamar (C4), lipod (C5), quinsol (C6), *b*alitaka

Table 1. Morphological characteristics of the blade of taro cultivars in the Bicol region, Philippines (2016).

Cultivars	Common	Color of the Blade			
		Dorsal	Ventral	Margin	Vein at the ventral part
C1	Unknown-1	Dark green	Pale green	Green	Purple
C2	Ito Ini	Dark green	Pale green	Green	Dark purple
C3	Duguan	Dark green	Pale green	Green	Light green with tinge of purple
C4	Sinamar	Dark green	Pale green	Green	Light green
C5	Lipod	Dark green	Light green	Purple	Light purple to dark purple
C6	Quinsol	Dark green	Light green	Pale green	
C7	Balitaka	Dark green with purple dot at the middle	Light green	Pale purple	Pale green with light purple veinlet
C8	Unknown-2	Dark green with purple dot at middle	Pale green	Green	Light green veinlet
C9	Unknown-3	Dark green with purple dot at the middle	Pale green	Purple	Purple
C10	Princesa	Dark green	Pale green	Pale brown	Pale green
C11	Binting Dalaga	Dark green	Pale green	Green	Pale green
	Tinahig	Dark green	Pale green	Purple	Purple
C12		with small purple dot at the middle			
C13	Agat paya	Dark green with small purple dot at the middle	Pale green	Very light purple	Pale green
C14	Inuroan	Dark green with small purple dot at the middle	Pale green	Green	Pale green
C15	Negrito	Dark purple green	Dark green	Dark purple	Dark purple
C16	Unknown -4	Dark green with small purple dot	Pale green	Purple	Pale green
C17	Kulduroy	Dark green	Pale green	Dark green	Green

(C7), prinsesa (C10), binting dalaga (C11), tinahig (C12), agat paya (C13), inuruan (C14), negrito (C15) and kulduroy (C17). There were four more distinct cultivars which were not given identity by the Bicolano taro farmers and this were considered in this study as unknown-1 (C1), unknown-2 (C8), unknown-3 (C9) and unknown-4 (C16). Each of the identified cultivar were noted to possess a set of characteristics unique to it. Color of the blade of the samples were analysed based the color of its dorsal, ventral, margin and vein at the ventral part .

Dorsal. Dorsal is the part of the leaves that is facing the soil, back side of the ventral. Dorsal color of the taro samples from the region were generally dark green particularly the C1, C2, C3, C4, C5, C6, C10, C11, C15 and C17. Some cultivars however were noted to have small purple dot at the middle including C7, C8, C9, C12, C13, C14 and C6.

Ventral. Ventral is the upper portion of the leaf, the one that is facing the sunlight. Color of the ventral of taro samples studied ranges from dark to pale green. Specifically it was noted that only C15 has a dark green ventral, thirteen others have pale green ventral including C1, C2, C3, C4, C8, C9, C10, C11, C12, C13, C14, C16 and C17. The other three possess light green ventral such as the C5, C6 and C7.

Margin. Leaf margin is the boundary area extending along the edge of the leaf. Margin characteristics of taro cultivars studied in the Bicol Region were either green or purple. There were cultivars with green margin such as C1, C2, C3, C4, C8, C11 and C14 while C17 showed a dark green margin. Those with purple were C5, C6, C7, C9, C12, C13, C15 and C16 with intensity that changes from dark, pale and very light. A cultivar, C10 appeared distinct from the rest because of its pale brown margin.

Veins at the ventral part. Samples showed either green or purple vein at the ventral part with varying intensities. Those with purple veins at the ventral includes C1, C2, C9, C12, and C15, with color intensity that ranges from dark purple to pale and light. Three cultivars reflected unique viens such as C5 having light to dark purple veins, while C3 and C7 indicated green with tinge of purple. The rest of the cultivars such as C4, C6, C8, C10, C11, C13, C14, C16, and C17 showed veins of green to light green.

Results showed that most leaves of taro is generally green on the dorsal part, this must be due to the bifacial structure of the leaf wherein only one surface is directly exposed to sunlight. In the ventral side of the blade is generally light green in color. The presence of purple pigment on the leaf vein at the ventral side of the blade for some cultivars served as basis for identifying the differences of the cultivars. Likewise, the presence or occurrence of purple pigment at the middle of the lamina was likewise considered for characterization since there are prominent differences on leaves of every cultivars in terms of the ventral veins. Figure 1 presents sample leaf for each cultivar noted in the Bicol Region.

Characteristics of the Petiole

A stalk that attaches the leaf blade to the stem is called a petiole, the length of the petiole determines the height of the taro plant. Each taro leaf is made up of an erect petiole and a large lamina.

Taro cultivars have varying petiole characteristics as indicated in the differences in the point, the colors vary its intensity from dark green to light green. Two other cultivars were noted with greater variation in its point of attachment to the blade such as C11 which is yellow green, and C13 is pink.

Base. Base of the petiole showed different characteristics among the sample cultivars subjected to

observations. Color comes in green, purple, pink and white. Those with green base such as C1, C2, C4, C5, C9, C12, C14, C16, and C17 showed intensities from dark to light to pale green. Cultivars with purple base were C3 and C15, C8 has a touch of purple and white. Pink base was likewise observed in C6 and C10, C7 has pale green and pink base.

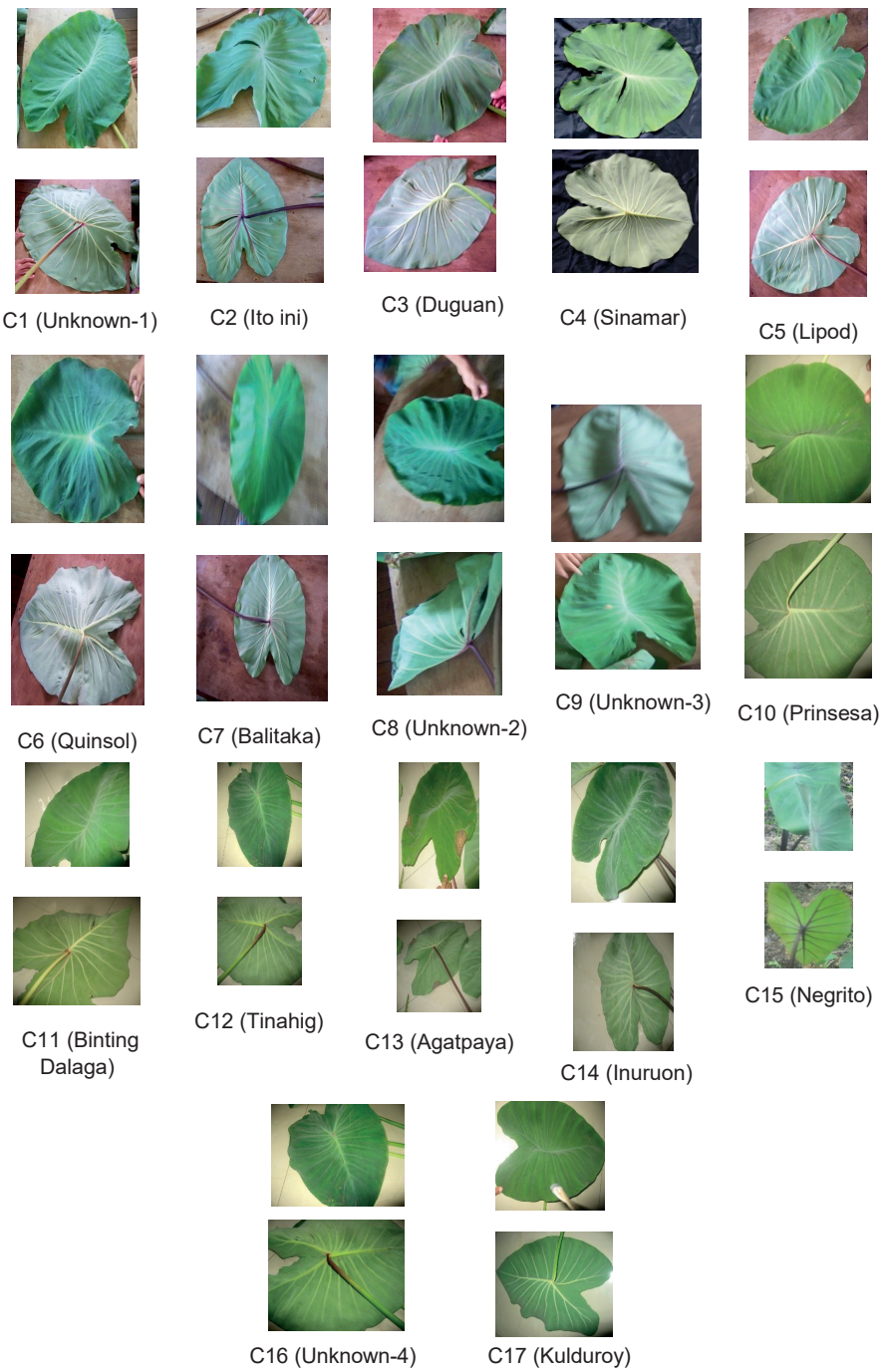

Fig. 1. Sample Leaf for Each Taro Cultivar in the Bicol Region, Philippines.

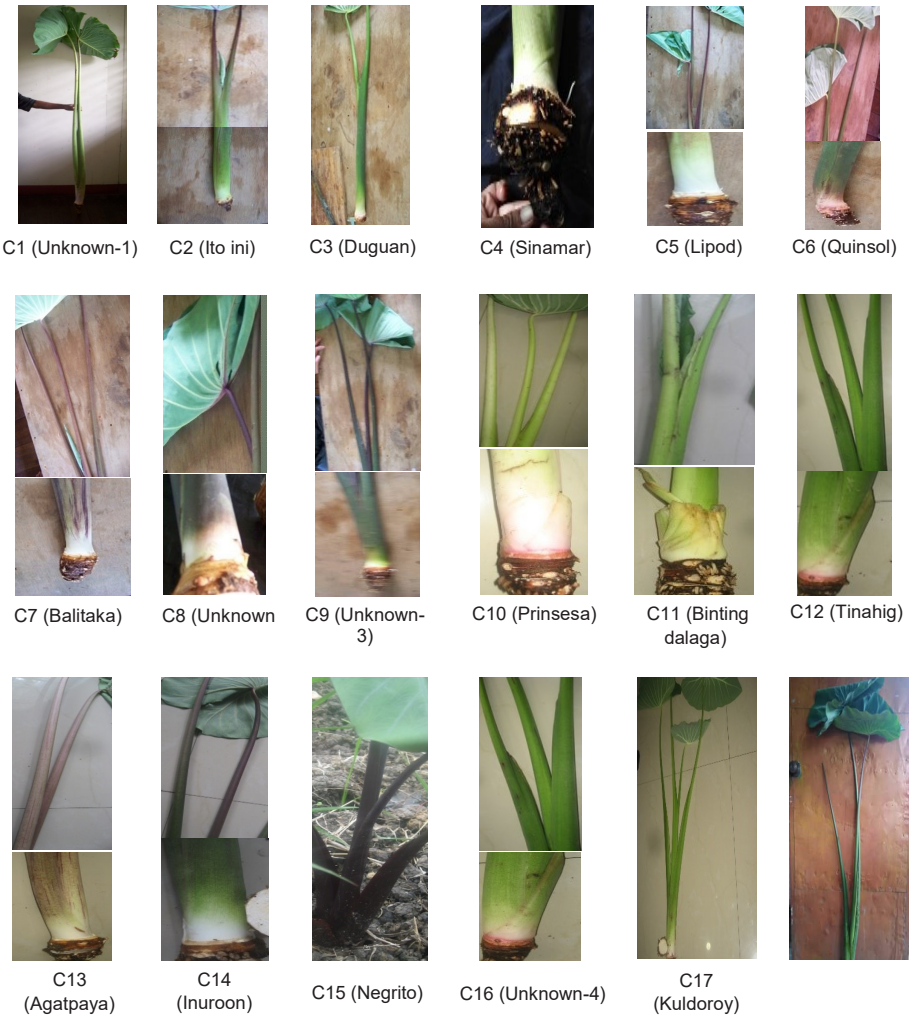

Fig. 2. Petiole of taro cultivars in the Bicol Region, Philippines.

Entire length of the petiole. was observed to show of attachment to the blade; its base and the entire length.

Point of attachment to the blade. The part of the petiole that is attached to the blade of the identified cultivars possesses color that may either be green, dark or light green, yellow green, purple and even pink. Cultivars with purple petiole were C1, C5, C7, C9, C12, C14, C15, C16, however of varying intensity as specified in Table 2. Similarly those cultivars with green point of attachment includes C2, C3, C4, C6, C8, C10, C17variations as either unicolor or with streak of other colors. Most unicolors were colored green, either dark or light. Cultivars noted to possess such characteristics were C1, C4, C6, C8, C11, and C14. Others showed a mix of colors as indicated in Table 2. Petiole of every cultivar is shown in Figure 2.

It can be noted in Table 2 that taro cultivars have varying petiole characteristics as indicated in the differences in the point, the colors vary its intensity from dark green to light green. Two other cultivars were noted with greater variation in its point of attachment to the blade such as C11 which is yellow green, and C13 is pink.

Base. Base of the petiole showed

Table 2. Petiole characteristics of taro cultivars in the Bicol Region, Philippines.

Variety	COLOR OF THE PETIOLE		
	Point of Attachment to the leaves	Base	Entire length
C1	Purple	Green	Green
C2	Dark green	Dark green	¾ of its length is purple
C3	Light green near to point of attachment	Light purple	Light purple from the base and green at the middle of its length
C4	Dark green	Dark green	Dark green
C5	Light purple	Green	Light purple to dark purple
C6	Dark green	Pinkish	Dark green
C7	Light purple	Pale green to pinkish,	Light purple with streak of dark purple along its length
C8	Dark green	Purple and white	Dark green
C9	Light purple	Light green	Dark green at the lower part & purple at the middle extending upward
C10	Pale green	Pink	Yellow green
C11	Yellow green	White	Green
C12	Purple	Pale green	Dark to light purple
C13	Pink	White	Pinkish with streak of purple
C14	Purple	Dark green	Dark green
C15	Dark purple	Purple	Dark purple
C16	Dark purple	Dark green	Dark green to purple
C17	Light green	Light green	Light green with streak of dark green along its length

different characteristics among the sample cultivars subjected to observations. Color comes in green, purple, pink and white. Those with green base such as C1, C2, C4, C5, C9, C12, C14, C16, and C17 showed intensities from dark to light to pale green. Cultivars with purple base were C3 and C15, C8 has a touch of purple and white. Pink base was likewise observed in C6 and C10, C7 has pale green and pink base.

Morphometric Characteristics

Morphometric is one important aspect that is looked into when a plant like taro is being characterized. In looking at the growth pattern, one cannot do away with measuring the plant height and other growth determining parameters. The morphometric evaluation is important to establish the claim of what cultivar to propagate when plant height of the cultivar is to be considered. Table 4 presents the morphometric characteristics of taro cultivars noted in the Bicol Region.

Petiole or stalk length. It can be gleaned from table 4 that height of taro ranges from 21 to 66 inches, revealing that it is not a tall plant. The shortest cultivar is C15 having an average petiole height of 21.2 inches, more than 40 inches lower than the tallest cultivar, C1. It can be inferred that C15 is just 1/3 in height of C1. All the rest of the cultivars have stalk length that is not so different from each other, of medium plant height of more or less 40 inches with an SD of 9.32.

Leaf blade. Morphometric of the blade includes the length and the

Table 4. Morphometric characteristics of different taro cultivars in the Bicol Region, Philippines.

Variety	Petiole or Stalk length (inches)	Leaf Blade		Corm	
		Length (inches)	Width (inches)	Length (inches)	Width (inches)
C1	66	28	21	8	3.0
C2	42.4	20	13	3.1	2.04
C3	45.4	17.2	12.4	7.3	2.75
C4	36	19.1	13.4	4	2.05
C5	38	17.4	12	5	2.36
C6	43.5	18.5	13.5	3.8	2.0
C7	38.2	16	12.2	3	2.1
C8	38.6	19	14	5.4	2.2
C9	46	18.1	12.9	4.5	2.5
C10	32.6	15	10.2	3	1.8
C11	39	15	10.5	4	1.75
C12	28.5	18.5	13.5	4	2.8
C13	46.5	17	12.6	3	2.3
C14	40.7	18	13.7	2.5	2
C15	21.2	12.5	8.4	1.2	.75
C16	35.5	12	9.2	3.8	2.5
C17	39	16	10	2	2.5
Mean	39.82	17.49	12.50	3.98	2.20
Standard Error	2.26	0.85	0.67	0.42	0.12
Standard Deviation	9.32	3.50	2.77	1.73	0.51

width. Data indicated that the length of the taro blade ranges from 12 to 28 inches, cultivars C15 and C16 revealing the shortest length and C1 showing the longest blade. All the rest of the cultivars are with length that are not so different from each other. It was however observed that cultivars with higher stalk length were also the ones with longer blade.

There is a positive correlation between the petiole and the leaf blade length given by r=0.7780 and also true with the petiole and leaf blade width as indicated by r=7925.

Width of the blade comes proportional with its length. Data showed that the width of taro blade ranges from 8 to 21 inches, C15 with the lowest width and C1 having the highest. This result is consistent with the findings for the stalk length and blade length.

Corm. Corm of the taro plant is not that big, however a good source of carbohydrate. Morphometric of the corm revealed that its length ranges from 2 to 8 inches and the width ranges from 2 to 3 inches. The cultivar with biggest corm is C1 and those with smallest corm is C15. Results is consistent with the findings for stalk length and leaf blade that those cultivars with longer stalk have longer and bigger blade. These cultivars were also the ones having big and long corm. Standard deviation of 1.75 and 0.51 for corm length and corm width , respectively indicated the consistency of data for corm. Correlation analysis further indicated that corm length and corm width is positively correlated with petiole length and width as proven by r=.6673 and 0.6208, respectively.

It can be noted that a tall cultivar of taro possesses the largest surface leaf area and the shortest plant height would also bear the narrowest leaf area. It implied that the leaf area is dependent on the plant height, and is the plant's mechanism to maintain a

balanced or proportioned plant growth. Moreover, statistical result on standard error showed that there was a minimal difference on the morphometric measurement except for a petiole length. Morphometric characteristic of taro cultivars post greater significance in the choice of what cultivar may be propagated for certain purpose.

Taro cultivars (Fig.3), based on their characteristics were associated by local farmers to certain utilization particularly in processing or cooking certain food products. Most lowland cultivars like C10, C5, C3, and C12 are efficient in leaf production and are the once used in cooking the commonly know “laing” of the Bicolanos. These are the varieties commonly found in Albay and Partido area, justifying for the involvement of these places in cooking “laing” either for personal consumption or for entrepreneurial purposes. The unknown varieties as claimed by some farmers and ordinary consuming individuals can also be utilized for cooking of Taro recipes.

Some cultivars can be grown in upland area such as C13, C7, C6, C10, and the 4 unknown varieties such as

Fig. 3. Corm of Taro Cultivars in the Bicol Region, Philippines.

C1, C8, C9 and C16. Most of these varieties are efficient in corm production as claimed by Nabua Taro farmers. Corm is also used in processing delicacies that are loved not only by local folks but also those from other places.

Taro Field Gene bank

Field genebanks (FGB) are commonly used for the conservation of taro genetic resources in the Pacific and other taro growing regions (Ebert and Wagainabete, 2018). Taro cultivars were noted to grow either in upland or lowland areas. It can be grown in all kinds of soil types but grows best in a friable loam soil, well-drained land. It can be planted in moist environment, in rice paddies and even in ones own backyard. Cultivars found in the Bicol Region are not so difficult to propagate because they all practically grow in all types of soil but there is one type of soil that a particular cultivar grows best. There were those that grow best in muddy or soil submerged in water while others are found in ordinary soil of the upland.

Field gene bank for taro established by this study were located on sites that were favorable for taro. Lowland varieties, were grown submerge in water particularly in a rice paddies or areas where the soil is always moist. The upland variety needed water occasionally during the period of its growth. All the noted taro cultivars were collected and planted in the established field gene bank to conserve its genetic resources.

Planting materials of 17 taro cultivars were collected, 50 seed pieces for each cultivar were planted in a designated area in the field genebank. One area of 50 plant per cultivar was maintained. Traditional farming system was employed in planting the seed pieces of taro with periodic weeding and cleaning of the plant where the old matured leaves were removed. Compost or organic fertilizer was occasionally applied to provide sufficient nutrient to the planted cultivars.

CONCLUSION

In the six provinces of the Bicol region, there were 17 distinctly different cultivars. These were identified *as* ito ini (C2), duguan (C3), sinamar (C4), lipod (C5), quinsol (C6), balitaka (C7), prinsesa (C10), binting dalaga (C11), tinahig (C12), agat paya (C13), inuruan (C14), negrito (C15) and kulduroy (C17). There were four more distinct cultivars which were not given identity by the Bicolano taro farmers and this were considered in this study as unknown-1 (C1), unknown-2 (C8), unknown-3 (C9) and unknown-4 (C16). Each of the identified cultivar was noted to possess a set of characteristics unique to it.

Most leaves of taro cultivar is green on the dorsal part, this must be due to the bifacial structure of the leaf wherein only one surface is directly exposed to sunlight. In the ventral side of the blade is generally light green in color. Purple pigment on the leaf vein at the ventral side of the blade for some cultivars served as basis for identifying the differences of the variety. Likewise, the presence or occurrence of purple pigment at the middle of the lamina was considered for characterization since there are prominent differences on leaves of every variety in terms of the ventral veins.

Colors of petiole of taro cultivars in the locale of study were different. It comes in green and purple of varying intensities or a combination of these colors. The colors are observed either in the whole length, at the base or at the point of attachment of the blade and the petiole. These characteristics together with the blade characteristics served as basis of identifying each of the taro cultivar in the region.

There is a very rich corm characteristics of taro cultivars in the

region which may be promising due to its significance in the production of flour. The flesh comes in either yellow, white or pinkish with fiber that can either be purple, dark yellow, brown or light orange. The varying colors of the corm may add value to the product/s because of its natural color. There was also a slight difference noted in the color of the corm skin of the various cultivars. They generally comes in brown but corm skin of some cultivars have a touch of purple or pink. Majority of the taro cultivars were of medium height, though a few is tall and erect. Cultivars with green stems are of medium height

Leaf area is dependent on the plant height, tall cultivar possesses the largest surface leaf area and the shortest plant height would also bear the narrowest leaf area. These cultivars were also the ones having big and long corm.

Field gene bank for taro established by this study were located on sites that were favorable for taro. Lowland varieties, were grown submerge in water particularly in a rice paddies or areas where the soil is always moist. The upland variety needed water occasionally during the period of its growth. All the noted taro cultivars were collected and planted in the established field gene bank to conserve its genetic resources.

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