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ENCAPSULATION OF PLANT GROWTH-PROMOTING BACTERIA USING VERMICAST AND COW MANURE FOR BASIL PLANT (*Ocimum basilicum*) GROWTH AND PLANT NUTRIENT ENHANCEMENT

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Abstract:

Enterobacter sp. bacteria was successfully selected from UITM Pathology lab collection with the specific characteristic which is IAA production, and phosphate solubilization, nitrogen fixation test, siderophore production, and cellulose degradation. The selection of *Enterobacter* sp. was referred from the potential amount of IAA production that suitable for basil plants that rich with chemical compositions. The bacteria was entrapped as a potential of plant growth-promoting bacteria that consisted with sodium alginate solution, vermicast and cow manure. The bacteria population for all encapsulation of biofertilizer is range from $10^8 - 10^{14}$.Vermicast and cow manure is act as carriers for the bacteria that has potential in a long lifespan. Three treatments which are encapsulated of vermicast, encapsulated of vermicast and cow manure, and encapsulated of vermicast and cow manure with PGPB were observed for the plant growth such as plant height (shoot length),number of leaves and plant nutrient enhancement of (*Ocimum basilicum*). This study observed that the treated plants with encapsulated PGPR with vermicast and cow manure showed the positive impacts on the growth and plant nutrient compared to control plants. Encapsulation technique is used as the method for entrap the bacteria with the carrier.

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Keywords:

Encapsulation, Bio-Fertilizer, Alginate, *Enterobacter*, *Ocimum Basilicum*, Vermicast, Cow Manure

Introduction

The basil plant (*Ocimum basilicum*) with the other name great basil is one of the types of culinary herbs that play an important role in Asian regions, especially in the cuisines of southeastern Thailand and the Vietnam. *Ocimum* has 150 species and belongs to the family *Lamiaceae* A large basil whose characteristics can survive in various environmental conditions, including water stress, which affect its growth and oil production. The plant *Ocimum basilicum* is one of the most important aromatic plants used to flavour food in traditional medicines. In addition, *Ocimum basilicum* was chosen as a model plant for growth enhancement to increase the quality for the use of pharmacological activities. *Ocimum basilicum* is used as a culinary herb and also has a number of pharmacological activities to prevent and treat cardiovascular disorders, diabetes, menstrual cramps, digestive disorders, neurodegenerative disorders and cancer and has also been reported for activities in antioxidants, antimicrobial and larvicides (Purushothaman et al. 2018). In addition, it has the greatest economic importance and is cultivated and used worldwide due to the ever-growing demand for its products on the national and international markets. The objectives of this study are to determine the effects of using vermicast and cow manure for PGPR encapsulation in Basil plant growth enhancement.

Nowadays, chemical fertilizers are widely used as a stimulant for improving plant growth. According to the World Data Atlas (WDA, 2020), fertilizer consumption in Malaysia in 2018 was, 2106.5 kg per hectare. By design, the use of inorganic or organic fertilizers focuses on high production. The addition of higher chemical fertilizers in the agricultural sector leads to environmental pollution that can harm the ecology of plants, animals, and humans. Excessive use of inorganic or chemical fertilizers can affect agricultural practices, thereby affecting the physical and biological health of arable land (Sofyan et al., 2019)**.** Fertilizers have been found to contain heavy metals like Mercury, Lead, Cadmium, and Uranium, which can disrupt the kidneys, lungs, and liver and result in cancer. 22 dangerous heavy metals, including silver, nickel, selenium, thallium, and vanadium, which are all directly associated with risks to human health, were found in over 29 well-known fertilizers (Thorat and More 2022)**.** Otherwise, to counter or overcome chemical fertilizer consequences, bio-fertilizer was focusing to be an important element in crop production to enhance plant growth without give harm to environment. Plant growth-promoting bacteria (PGPB) are the greatest agronomic practices in agriculture sector (Amalero et al. 2003). Bio-fertilizers are living microbial inoculants that are added to the soil to improve the plant growth (Riaz et al. 2020). In addition to applying the beneficial bacteria especially bacteria, encapsulation is technique that act as protection or capsule medium for the microorganism. According to Bashan (2016), the encapsulation procedure was introduced with the benefits of protecting beneficial microorganisms over a long-term storage duration. Sodium Alginate ($C_6H_9NaO_7$) is one of the polymers that have potential for microbial carrier in encapsulation technique.

Vermicast and cow manure in bacteria encapsulation is specifically for improving the lifetime of bacteria in beads and soils. Nutrient content, primarily nitrogen, phosphorus, and potassium, is important as it affects soil applications rates and treatment techniques. With the

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characteristics of vermicast and cow manure, it has the potential to support the lifespan of bacteria and at the same time can increase the availability of nutrients in the soil and for the plant.

Literature Review

Six points of literature review (LR) were conducted on this topic in this paper: The first will be the production of basil plants; the second will be the effect of biofertilizer on basil plants: Third, efficient microorganisms (EM) as biofertilizers in agricultural production: fourth, approaches to biofertilizer formulation: Fifth, microorganism encapsulation: finally, microorganism encapsulation materials.

Basil Plant Production

Basil plant (Ocimum basilicum) is a culinary herb that produce compounds that are beneficial to the health of people and other animals. (Mohamed et al., 2016). According to WHO (2008), medicinal and aromatic plants are used by 80 percent of the world's population for medicinal and therapeutic purposes. Basically, Ocimum basilicum is a remarkable medicinal plant that has been used widely in traditional and modern medicine to treat headaches, coughing, diarrhea and other diseases. It has numerous applications in the food, pharmaceutical, dental, fragrance, and cosmetic industries. Basil is a very aromatic herb that is used to flavor dishes, sauces, marinades, cheese, and meat products, as well as in traditional treatments (Frąszczak et al., 2015). Most of these preparations are used to treat chronic health problems and moderate ailments by impoverished urban residents who do not have the financial means to purchase traditional pharmaceuticals from pharmacies (Madaleno, 2015). Within the species Ocimum basilicum, there are several variations that differ not only in basic morphological structure, but also in essential oil content and composition. The composition of the essential oil is determined by hemotype of affiliation and the basis for hemotaxonomy inside of highly polymorphic genus Ocimum and species Ocimum basilicum (Beatovic et al., 2015). The generation of essential oils Basil plant is not 7 solely dependent on plant genetics or developmental stage. The environment and its modifications can have a considerable impact on biochemical pathways and physiological processes that alter plant metabolism and, as a result, essential oil biosynthesis (Sangwan et al., 2001).

The Effect of Biofertilizer on Basil Plant

Unconventional efforts have recently been undertaken to reduce the amounts of chemical fertilizers applied to medicinal and aromatic plants to reduce production costs and environmental pollution without reducing output. Using soil microorganisms and biofertilizers to deliver nutrients to crops is one strategy for optimizing agricultural production with less use of chemical fertilizers while maintaining environmental health. (Khalediyan et al., 2021). Bio-fertilizers are less harmful to the environment than chemical fertilizers and help to reduce the usage of chemical fertilizers. As a result, the tendency presently is to use bio and organic fertilizers. Basically, biofertilizers are microbial inoculants made up of livings cells of microorganism such as bacteria, algae, and fungi that can assist increase crop output. According to Kibret (2014), biofertilizers can influence plant growth directly through the production of phytohormones such as gibberellins, cytokinin's, and IAA, which act as growth regulators, and indirectly through nitrogen fixation and the production of biocontrol agents against soil-borne phytopathogens, which increases the formation of metabolites that encourage vegetative growth and the meristematic activity of tissues to produce more growth. The findings can be summarized with the utilizing different rates of organic manure

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independently or in combination with varying concentration of fertilizer enhanced vegetative growth and oil yield when compared to the control. 8 Resulting that the most significant increases in the main chemical components of leaf essential oil such as estragole, eucalyptol, linalool, and trans-4- methoxycinnamaldehyde were obtained by utilizing organic fertilizer at $60m3$ /fed that combined with 2.0 and 4.0 of biofertilizer (Khalediyan et al., 2021). Other than that, reported that the application of biofertilizers significantly increased most traits of basil, including fresh and dry shoot yield, dry leaf yield and leaf area index (Jahan et al., 2012). It has been observed that dual inoculation with *Azotobacter* sp., *Azospirillum* sp., phosphate rock, inorganic nitrogen, and mycorrhizae promoted the growth of Datura stramonium and Amni visnage Fam (Tahami et al., 2017).

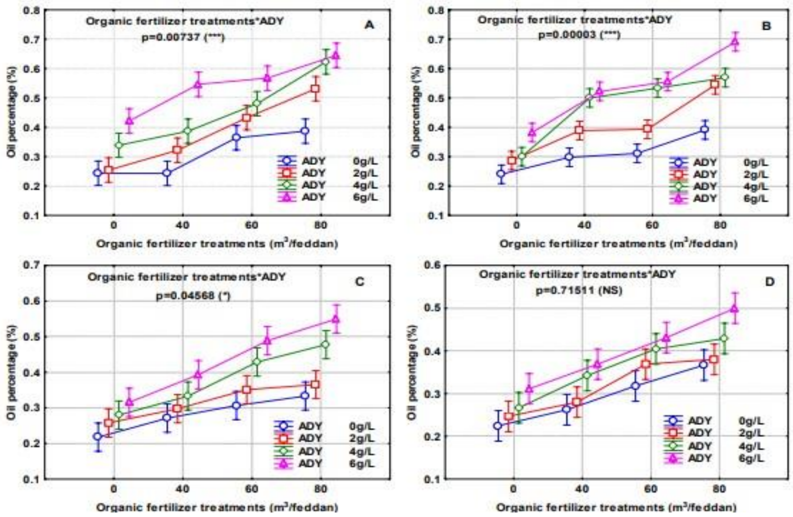

Figure 1 The Effect of Organic Fertilizer and Active Dry Yeast Treatments on the Oil Percentage (%) of *Ocimum basilicum*

Effective Microorganims (EM) as Biofertilizer in Agriculture Production

Effective microorganisms (EM) are made up of a broad variety of bacteria, yeasts, and fungus with provide adaptability in terms of the vast range of applications that is may be utilized on. EM also is used in a variety of agricultural and environmental management system. Moreover, EM is a green technology that consists of a fermented mixed culture of coexisting and mutually compatible microorganisms in an acidic medium. Plant-growth promoting bacteria and bio stimulants have been proven to increase plant nutrient uptake, growth, and yield through several underlying mechanisms such as changes in soil structure, nutrient solubility, root growth, plant physiology, and symbiotic relationships (Iriti et al., 2019). Effective microorganisms are a soil inoculant that is promoted as a crop production enhancer. In agriculture sector, biofertilizer is widely used as the main element to sustain the soil fertility, plant physio-chemical and others. Moreover, plant vigour and survival are increased by complex interactions between soil-borne microbes such as rhizobacteria, rhizobia and endophytic fungi and plants via direct and indirect mechanisms. Soil bacteria can directly create symbiotic relationships with plants to boost nutrient absorption and plant vigour (Pineda et al., 2010). To sustain the soil fertility, the usage of chemical fertilizer in agriculture production is important to be minimized. Waterway pollution, chemical burn to crops, increased air pollution, acidification of the soil, and mineral depletion of the soil are the variation of the problems that caused by chemical fertilizer. Contrary to popular assumption,

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chemical fertilizers do not assist replenish soil nutrients and fertility rather they 10 replenish just nitrogen, potassium, and phosphorus. Inadequate N delivery frequently leads in plant with slow growth, low protein levels, inadequate yield of low quality, and wasteful water use (Hayat & Ali, 2010). Even though, chemical fertilizer is one of the important elements in agriculture productions, it contributes detrimental effects on soil and plant growth. Despite this, to overcome the huge chemical fertilizer usage on agriculture production, EM as biofertilizer is one of the solutions that can replace chemical fertilizer application. Likewise, biofertilizers have various advantages over chemical fertilizers, including the fact that they are non-polluting, affordable and use renewable resources. (Olle & Williams, 2015). (Table 1)

Table 1: List of Effective Microorganisms, Types of Plant and Benefit Towards Plants

Effective microorganisms (EM)	Plant	Improvement of plant	Citation
Acinetobacter pitti	Rice	Increase plant height and yield	Ashfaq *et al., 2020*
Stenotrophomonas maltophilia	Peanut	Increase the plant growth under Nitrogen deficit condition.	Alexander *et al., 2019*
Azotobacter sp	Rice	Increase the plant height and yield in the field condition	Banik *et al., 2019*
Bradyhizobium japonicum	Soybean	Increase the plant growth and root system.	Egamberdieva *et al., 2018*

Biofertilizer Formulation Approaches

Microorganisms' applications as bioinoculants in agriculture is a critical activity with a growing requirement. Scientific studies, it is the addition of agriculturally beneficial microbes to the soil in order to achieve agricultural sustainability. Nutrients are important thing for plants to perform their growth enhancement. In the same way, there is a growing trend of utilizing multiple bioinoculants to improve soil health and crop output. The concept of inoculation is more like a mother recipe, transferring one generation to the next from time immemorial. Indeed, it was the equally scientific bioformulation containing an inoculant (Rhizobium), a transporter which means soil particles, a cell protectant (soil colloids) and moisture that allowed live cells to survive. (Singh et al., 2016) Furthermore, the effectiveness of bioinoculant technology is determined by two factors which are the microbial strain and the formulation of the inoculant. In addition, many different types of bioinoculants are utilized in agriculture, such as N fixers, P solubilizers, P mobilizers, biocontrol agents, PGPR, and other else. To be used, these bioinoculants must be placed in a carrier, either liquid or solid, along with osmoprotectants, sticking agents, nutrients, and other ingredients that resulting assembly is known as a bioformulation. (Singh et al., 2016). Other than that, a formulation may alter depending on the bioinoculants use, the types of soil, the types of plant, the nature of the application and the access to resources. The scientific literature on formulation development is

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limited and fragmented (Xavier et al., 2004). It because the majority of them are simply comparative studies of various 12 carriers, and the methodologies were more agronomic than bioengineering based. According to the accessible bibliographic database, more significant work has been done on the production of better strains using various methodologies. Nevertheless, numerous improved strains have been produced and granted patents in many industrialized nations, but they have yet to appear on the commercial market, possibly due to the ineffective formulation technology utilized for them (Brahmaprakash & Sahu, 2012). (Table 2)

Consideration	Citation
1. At room temperature, the inoculant formulation should have a sufficient shelf life.	Bashan*, 1998*
2. The inoculant should be simple to make and mix using existing industrial methods, and it should allow for the addition of nutrients.	Bashan*, 1998*
3. The inoculant must compete for nutrients and livable niches with native soil microorganisms, as well as survive against grazing protozoa.	Bonkowsk*i, 2004*
4. Polymer-entrapped compositions must be non-toxic and free of preservatives that could harm the inoculant microorganism.	Deaker *et al., 2004*

Table 2: Qualities of Model Bioformulation That Can Be Considered.

Encapsulation of Microorganism

Encapsulation of beneficial plant microorganism has been an efficient technique in the agriculture industry. This procedure involves the use of a scaffold or a tissue to protect the microbial cell from the environment. According to Prasad (2017) The encapsulation in agriculture is to obtain a surrounding structure that protects, releases, and functionalizes microorganisms. Other than that, encapsulation is a process where a substance is trapped in a capsule that contain microorganisms. The goal is to keep the microorganisms from developing resistance to certain conditions. To keep the microbe alive, it can also be encapsulated to shield the organism from changes in the external environment such as the hostile soil environment. The majority of this is to entrap the microorganism for an extended length of time in order to generate the crop with the imprisoned life material. Bacterial encapsulation has a lengthy shelf life and can even be stored at room temperature for extended periods of time (John et al., 2019a).

Materials for Encapsulation of Microorganism

Generally, encapsulation technique is a process to entrap a substance which is microorganism with another material as a wall material. There are various materials that can be utilized to encapsulate that act as shell for the microorganism. It is critical to guarantee that the conditions. are not toxic to the microorganisms, taking into consideration that bacteria might be impacted not only by the materials used to make the capsules, but also by other solvents, such as alcohols or acetone. (Šipailienė & Petraitytė, 2018). Furthermore, important factors include which microorganisms will 14 be entrapped, the environment in which the capsules will be used,

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which method is preferred as the cell release mechanism, and other factors that may affect encapsulation efficiency and cell viability during the process as well as after storage.

Sodium Alginate

Alginate is a polymer that occurs naturally as a structural component in marine brown algae (*Phaeophyceae*) and as a capsular polysaccharide in soil bacteria. Sodium alginate also well-known as elements of the hydrogel category. Basically, the hydrogel is a cross-linked polymeric network that is water-swollen and formed by the simple reaction of one or more monomers. Sodium alginate also the most often utilized substance for the bio-encapsulation of microorganisms. Ionic gelation is used to create calcium alginate beads or capsules by dropping an alginate solution into a calcium chloride solution. Then, sodium alginate is a polymer that generated from brown algae that gels when it encounters calcium chloride. The sodium alginate solution will be combined with the cell culture which is microorganism before being placed into the calcium chloride solution at concentrations ranging from 0.05 M to 0.1 M.

Vermicast

Basically, earthworm feeding, and casting activities deposit organic residues into the soil, which helps nutrient cycling and soil structure development. Earthworms consume vermicast, which is more stable than soil and contains certain inorganic and organic components, enzymes, bacteria, and plant growth hormones obtained through 15 the transit of soil aggregates through the earthworm’s intestines (Teršič & Gosar, 2012). Furthermore, through a mutualistic connection with soil microorganisms, may consume and transform low-quality organic materials into a nutrient-rich product. Previously studies, compared the physiochemical properties of vermicast and soil from an agricultural field and a botanical garden, as well as the effect of various agricultural management practices such as ploughing and the application of chemical fertilizers and pesticides on earthworm activity. Resulting, pH, total dissolved solids (TDS), EC, Ca, Na, Li, K, and heavy metals were lower in the vermicast whereas organic carbon (OC), P, and N were higher in the vermicast than in the corresponding soil (Singh et al., 2016). The genotoxicity reduction was confirmed by vermicast samples from both the agricultural field and the botanical garden when compared to agriculture and botanical garden soil, respectively (Singh et al., 2018). The reduction of genotoxicity by earthworms was greater in the botanical garden than in the agricultural land due to the increased population of earthworms in the botanical garden soil compared to the agricultural land soil.

Cow Manure

Application of organic amendments such as livestock manures were reported to increase the content and availability of SOM (Li et al., 2019). Basically, organic supplements can improve the availability P in the soil by modifying the pH of the soil and the activity of nutrition cations such as $Al3+$, $Fe3+$, and $Ca2+$. For a long time, the cow manure was also applied to the soil as a classic additive for nutrients and the improvement of soil quality (N et al., 2015). Another source of organic fertilizers are 16 fruit and vegetable wastes which support microbial life during transfer to soil for PGPR formulation which also maintain a moisture content of as well. as soil pH and soil retention capacity water (Danish et al., 2019). However, if animal dung could be efficiently moved to nearby crops and used as plant fertilizer, pollution and contamination would be reduced, changing manure from a waste to resource. Manure utilization as a source of macro and micronutrients in agriculture is an ancient practice that, if done sustainably and without overapplication, can help to address this nutrient management conundrum. According to malomo et al., 2018, the use of animal dung as fertilizer has the

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potential to minimize the environmental effect of the livestock industry, farmer fertilizing costs, farmers reliance on mineral and synthetic fertilizers.

Materials and Methods

Selection of Plant Growth-Promoting Bacteria (PGPB)

Bacteria was selected under UITM Pathology Lab collection. Isolate *Enterobacter* (SA-10) was selected to be encapsulated.

Encapsulation of Vermicast and Cow Manure as Carrier for Bacteria (Enterobacter)

Encapsulation technique used was modified according to Bashan (*1986*). Liquid nutrient broth was prepared as transfer medium for bacteria cultured, and the model of bacteria is SA-10 (*Enterobacter* sp.). SA-10 were grown for 24 hours at 30 ˚C in incubator shaker. Bacterial entrapment within beads was formed in a laminar flow under sterile circumstances. 500 ml of bacterial culture with aseptic condition was combined with 2 % Sodium Alginate powder and gently stirred for one hour. Next, the mixtures of Sodium Alginate, bacteria, vermicast, and cow manure were pull up using 20 mL syringe into solution 0.1 M Calcium dichloride, CaCl2. Beads formed immediately in the Calcium dichloride, CaCl2 solution and the beads were left in the solution for ten minutes. Then, the beads will immediately form in the Calcium dichloride, CaCl2, solution. The beads were removed from the solution and cleaned twice with sterile tap water before drying for one or two hours. Lastly, the beads were kept in sterile Ziplock bags and kept in chiller at 5 ˚C.

Encapsulation Bio-Fertilizer (Without Bacteria)

The similar method for encapsulation Bio-fertilizer without bacteria mixture that proposed by Bashan (1986). The entrapment process of vermicast and cow manure were conducted under sterile conditions in a laminar flow. 2 % sodium alginate powder was mixed with one L distilled water and stirred gently for 45 minutes to one hours. Next, the mixture of sodium alginate and distilled water was mixed with vermicast, and cow manure. The mixture of vermicast and cow manure was drawn using syringe into solution 0.1 M Calcium dichloride, CaCl2. Beads formed immediately in the Calcium dichloride, CaCl2, solution and left it for ten minutes. The beads were washed twice with sterilized tap water and then dried under the laminar flow for one to two hours. Lastly, the beads were rinsed with sterilized water for three times and kept in sterile zip lock bags and kept in refrigerated at 5 ˚C.

Determination of Bacteria Population

Firstly, one g of gel beads for each treatment were collected and moved into 9mL water and centrifuge at 20000 rpm. After that, one mL of the first suspension was taken and transferred to fresh nine mL of sterile distilled water and was shaken again. This step successfully established a 10^2 dilution. The procedure was repeated until a dilution of 10^{14} was reached. Then, drop plate method was done before calculation.

Total number of bacteria = $\left(\frac{Number\ of\ CFU \times 10^x}{Volume\ initial\ (ml)}\right) \frac{0.1}{0.1}$.

Plant Growth Parameters Data Collection

The model plant organism in this study was basil plant (*Ocimum basilicum*). Basil seeds were soaked in tap water for 24 hours with room temperature condition. Then, the seed germinated in a tissue towel. The growing seeds were moved to the seedling tray until they were ten days

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to achieve the maturity from seed. After ten days, the plants were transferred to a poly bag with biofertilizer (beads) treatment. The soil that uses for planting purpose collected from the farm and sieved to eliminate any gravel or other debris. The media was placed in an 8x12 inch poly bag with a total of 16 plants.

This study used a completely randomized design (CRD) with four treatments and four replications. For the experiment, a total of 16 pots were used. The basil plant was grown as a control, encapsulated biofertilizer with bacteria and without bacteria. The treatments were conducting ten g beads a pot for encapsulated biofertilizer with bacteria and ten g a pot for encapsulated biofertilizer without bacteria. After that, the plants were watered day by day in the early morning and late afternoon to ensure a continuing of the plant growth. Lastly, plant height, number of leaves per plant, fresh and dry weight were collected and measured.

Plant Nutrient Tissue Extraction and Analysis

Dry Ashing method was conducted to determine the total nutrient in leaves samples. Basil plant leaves were collected and placed in oven for leave's drying process at 60 ˚C for 24-48 hours. After the drying process was done, the leaves of basil were grinded using a sample grinder to get fine texture. 0.5 g of the dried samples were placed into a crucible for ash process at 300℃ for one hour, and the temperature was increased to 550℃ for another seven hours. For the next step, few drops of distilled water were added into the samples and another two ml of concentrated Hydrochloric acid (HCl) was added. The samples were left evaporated for ten minutes. ten mL of 20 % of nitric acid (HNO3) were added into the samples. The prepared samples were placed in a water bath at 100℃ for 30 minutes. Then, the samples in the crucibles were diluted with distilled water to 100 mL using a volumetric flask. Lastly the samples were filtered into a 100 mL plastic vial and ready for ICP test to analyse the nutrients content.

Statistical Data Analysis

The experiment was generated with four replications with completely randomized design. The SPSS application was used for data analysis, which included one-way ANOVA and comparing treatment means with Duncan test at 95 % probability.

Result

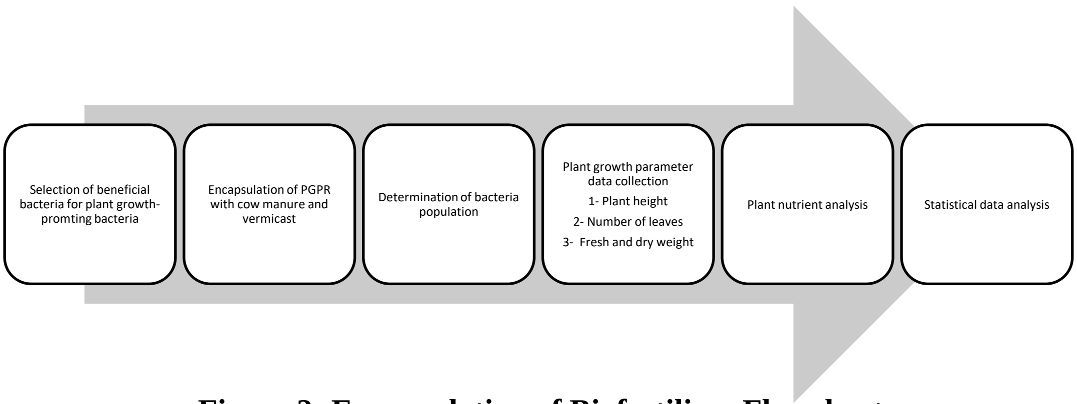

Figure 2: Encapsulation of Biofertilizer Flowchart

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Biochemical Properties of Bacteria Isolates

The biochemical properties of bacteria isolated is tabulated in table 2. The highest of zinc oxide solubility index at 41.66% is SA-8. Isolated SA-8 showed the highest of zinc carbonate solubility index at 50%. Other than that, the highest of phosphate solubilization ability is SA-8, SA-10, and SA-17 at 50%. Isolated SA-9 showed the highest of potassium solubilization. ability at 90.90%. In nitrogen fixation and siderophores production, all the isolated bacteria showed the positive results. For cellulose degradation, the positive results showed that only five isolated bacteria which are SA-9, SA-10, SA-14, SA-17, and SA-20. The indole-acetic acid (IAA) showed that the SA-10 is the highest compared to others. As a result, SA-10 was chosen to be encapsulated as a potential bio-fertilizer. (Table 3)

Table 3: Biochemical Properties of Bacteria Isolates (Nordin, S.A.S & Othman,N.M.I, 2021)

Isolates	Solubilization index ZnO (%)	Solubilization index $ZnCO_3$ (%)	Phosphate solubilization ability (%)	Potassium solubilization ability (%)	Nitrogen fixation	Siderophores production	Cellulose degradation	IAA production (mg/L)
SA-8	41.66. a	50.00a	50.00a	81.81b	+	+	-	2.559b
SA-9	19.04. b	40.00b	31.57d	90.90a	+	+	+	2.099cd
SA-10	13.04.c	38.46c	50.00a	76.92c	+	+	+	4.007a
SA-14	20.00. d	26.66d	40.00c	66.66d	+	+	+	1.901d
SA-17	31.25. e	43.75b	50.00a	90.00a	+	+	+	2.822b
SA-20	21.24. f	28.57d	47.61b	80.00b	+	+	+	2.23c

The Range of Bacteria Population (CFU/mL)

Table 2 showed the range of bacteria population in colony forming unit per millilitre is from 10^8 - 10^{14} CFU/mL for all treatment of encapsulation of biofertilizer. (Table 4)

Table 4: The Range of Bacteria Population (CFU/mL)

Treatment	Range of Bacteria Population (CFU/mL)
T1 (Encapsulated of vermicast) T2 (Encapsulated of vermicast and cow manure) T3(Encapsulated of vermicast and cow manure with PGPB)	10^8 – 10^14 CFU/mL

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Plant Nutrient Tissue Analysis

The macronutrient and micronutrient contents in the basil plant after treatment applications is tabulated in table 6. The highest phosphorus, calcium, magnesium, natrium for macronutrient content in the plant is T3 which is encapsulation of vermicast and cow manure with PGPB. For potassium content in plant, showed T2 (encapsulation of vermicast and cow manure) and T3 (encapsulation of vermicast and cow manure with PGPB) are the highest compared to others. In micronutrient content, showed that T3 which is encapsulation of vermicast and cow manure with PGPB is the highest in iron, zinc, copper, and manganese. (Table 5)

Table 5: Mean Value for Macronutrient and Micronutrient of Different Encapsulation Treatments. (T0 = Control, T1 = Encapsulation of Vermicast, T2 = Encapsulation of Vermicast and Cow Manure, T3 = Encapsulation of Vermicast and Cow Manure With PGPB)

Treatment	P (mg/kg)	K (mg/kg)	Ca (mg/k)	Mg (mg/k)	Na (mg/kg)	Fe (mg/kg)	Zn (mg/k)	Cu (mg/kg)	Mn (mg/k)
T0	61.33b	128.43b	30.40b	18.85b	79.52c	BDLb	BDLb	BDLb	BDLb
T1	12026.66ab	49387.33a	19172.66a	5451.83a	392.26b	315.70b	54.58a	12.03ab	155.33a
T2	11330.66ab	56063.33a	19390.00a	5618.33a	514.86ab	750.00b	87.50a	15.61a	144.16ab
T3	17846.66a	56063.33a	20270.00a	8041.66a	707.43a	2097.66a	96.10a	21.28a	169.70a

Growth Performance Of Basil Plant

Plant Height

Overall, the treatment of encapsulation showed the increasing in plant height from week 1 till week 4. The treatment encapsulation of vermicast and cow manure with PGPB (T3) showed highest in plant height at week 4 where the mean value was 35.43cm. Control treatment (T0) produced lower plant height at 17.05 cm. Furthermore, encapsulation of biofertilizer has significantly difference between week 2 to week 4. There is no significant difference at week 1 between all treatments because it was still early for the effect of biofertilizer. (Figure 3)

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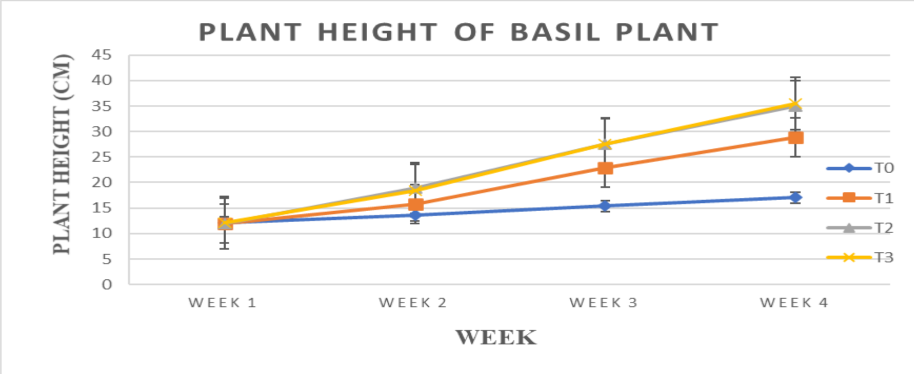

Figure 3 Mean Value for Basil Plant Height of Different Encapsulation Treatments. (T0 = Control, T1 = Encapsulation of Vermicast, T2 = Encapsulation of Vermicast and Cow Manure, T3 =Encapsulation of Vermicast and Cow Manure With PGPB)

Number of Leaves

Overall, the treatment of encapsulation showed the increasing in number of leaves from week 1 till week 4. The treatment encapsulation of vermicast and cow manure with PGPB (T3) showed highest in plant height at week 4 where the mean value was 398.25. Control treatment (T0) produced lower number of leaves at 238.25. Furthermore, encapsulation of vermicast and cow manure with PGPB (T3) showed the significant difference from week 2 to week 4 compared to other treatments. There is no significant difference between all treatments from week 1 till week 4 because it was still early for effect of biofertilizers. (Figure 4)

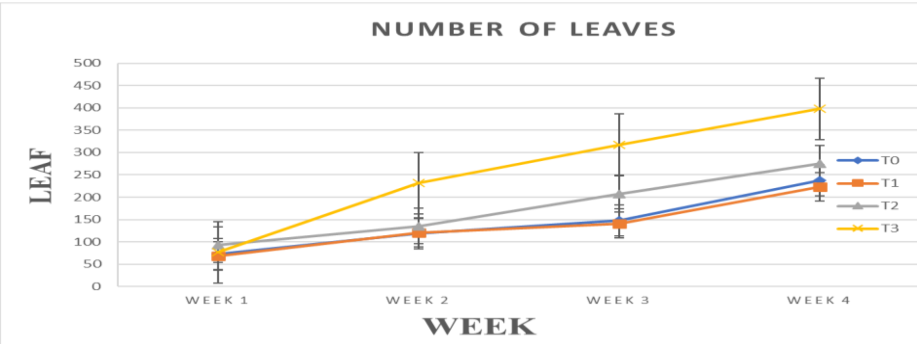

Figure 4 Mean Value for Number of Basil Leaves of Different Encapsulation Treatments. (T0 = Control, T1 = Encapsulation of Vermicast, T2 = Encapsulation of Vermicast and Cow Manure, T3 = Encapsulation of Vermicast and Cow Manure With PGPB)

Fresh Weight

The treatment with encapsulated of vermicast and cow manure produced highest of fresh weight compared to other treatments where the mean value was 29.28g. Control treatment showed the lower fresh weight where the value was 11.24g. The means of treatments showed the T3, T2, and T1 have significant difference between T0 (Figure 5).

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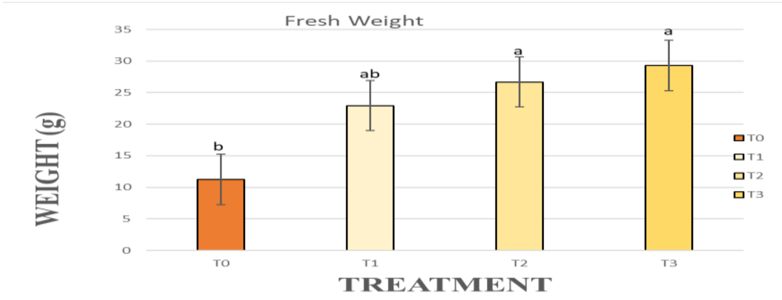

Figure 5 Mean Value for Fresh Weight of Different Encapsulation Treatments. (T0 = Control, T1 = Encapsulation of Vermicast, T2 = Encapsulation of Vermicast and Cow Manure, T3 = Encapsulation of Vermicast and Cow Manure With PGPB).

Dry Weight

The treatment with encapsulated of vermicast and cow manure produced highest of dry weight compared to other treatments where the mean value was 4.00g. Control treatment showed the lower fresh weight where the value was 2.15g. The means of treatments showed no significant difference between all treatments (Figure 6).

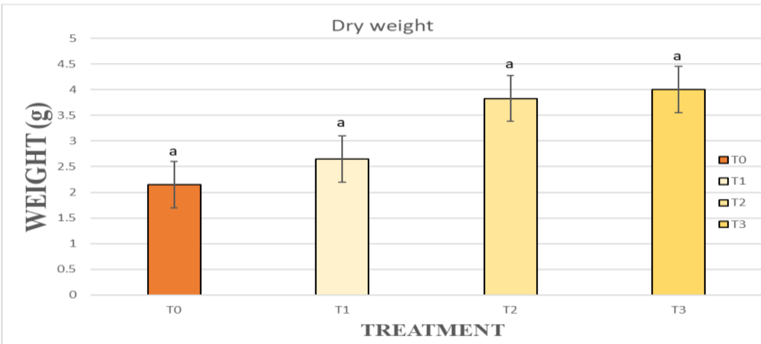

Figure 6 Mean Value for Dry Weight of Different Encapsulation Treatments. (T0 = Control, T1 = Encapsulation of Vermicast, T2 = Encapsulation of Vermicast and Cow Manure, T3 = Encapsulation of Vermicast and Cow Manure With PGPB).

Performance of Basil plant

The growth performance of basil plants was observed based on parameters such as plant height (shoot length), leaf number of plant, fresh weight, and dry weight after 4 weeks of treatment applications. The significance difference in treatment effects was using one-way ANOVA and the means of treatments were compared using the Duncan test. (Figure 7)

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Figure 7 Performance of Different Encapsulation Treatments. (T0 = Control, T1 = Encapsulation of Vermicast, T2 = Encapsulation Of Vermicast And Cow Manure, T3 = Encapsulation Of Vermicast And Cow Manure With PGPB).

Discussion

Bacteria isolates all have their own biochemical characteristics that can influenced the plant growth. In addition, microorganisms produce biochemical substances that significantly change the soil environment. Production indole acidic acid (IAA), gibberellins (GA), and cytokinin (CK) like substances, play regulatory functions in plant physiological processes (Raaijmakers et al. 2008). Based on (Table 3), IAA production from SA-10 is the highest one compared to others and it important to chemical compounds in basil plants. IAA and BAP caused an increase in the yield of essential oil (0.30% and 0.32% respectively) without much influence on the main compounds, but with some change in the composition such as the appearance of (germacrene-D) and the disappearance of (aristolene) (Hazzoumi et al., 2014). The selection of SA-10 with the highest of IAA production is essential for improving the chemical composition in basil plant especially the essential oil content. However, the bacteria population for all treatments is ranging from $10^8 - 10^{14}$ that has similarity to the large number of bacteria in the soil. There has been evidence for some time that soil contains many bacteria typically 10^8 - 10^9 cells per gram of soil and that the proportion of culturable bacterial cells in soil is only about 1% of the total number of cells in soil (Schoenborn et al. 2004). The encapsulation of biofertilizers is capable to supply a lot of bacteria population in the soil that can influenced the plant growth.

From the plant nutrient analysis (Table 5), showed that the encapsulation of vermicast and cow manure with PGPB is the highest in macronutrient and micronutrient compared to the other treatments. It also showed the significant difference in plant nutrient content of encapsulation of vermicast and cow manure with PGPB compared to the other treatments. The data showed that the combination of vermicast and cow manure as carrier for bacteria is effective to multiply the nutrient content that can use by the plant to grow. The grain nutrients analysis data showed that the inoculation of PGPB, either alone or in consortium, significantly increased grain phosphorus, and calcium uptake (Tsegaye et al. 2021). PGPB also increased the concentration of macro and micronutrients in plant leaves, including N, P, K, Ca, Mg, Fe, Mn, Zn, and Cu (Helaly et al. 2020). Evidently, these current studies showed the similarity of plant nutrient content enhancement in macro and micronutrients.

Plant height and number of leaves showed that the encapsulation of vermicast and cow manure with PGPB was significant difference between other treatments started from week 2 till week 4 (Figure 3 & 4). But there are no significant differences between all treatments at week 1

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because it was still early to show the effect of encapsulation of biofertilizer. This is because of the encapsulation of vermicast and cow manure with PGPB has a relationship in the soil after the treatment applied to supply nutrient to plant for growth performance. It is speculated that supplementing the gel beads with nutrient compounds will enhance their stability, provide protection and nourish the encapsulated cells (Young et al. 2006). For this current study, it has similarity with the study that conducted by (John et al., 2019).

On the other hand, there was no significant difference between all treatments of encapsulation of biofertilizer by using Duncan test (Figure 6) because the bacteria isolated which is *Enterobacter* probably no beneficial effects on dry weight of basil plants. But for fresh weight of basil plant still has significant difference for T2 and T3 (Figure 5) compared to control. However, this selected bacteria still influence plant height, number of leaves and fresh weight. of basil plants for physical parameter. Plants typically generate hormones such as auxin, which are utilized in the regulation of plant stem lengths, and IAA, which stimulates greater root length via PGPB formulation.

Conclusions

In a nutshell, the selection of bacteria which is *Enterobacter* is a suitable for biofertilizer on growth performance of basil plants. The highest IAA production at 4.00 (mg/L) is the important biochemical properties for PGPR that can influenced the chemical composition and essential oil in basil plants. Other than that, the range of bacteria population which is $10^8 - 10^{14}$ due to the variability of the bead sizes had a good potential for plant nutrient uptake. In addition, encapsulation of vermicast and cow manure with PGPB showed that the macro and micronutrients in basil leaves is higher compared to others. This means by the encapsulation with PGPB was successful compared to the others. Plant height and number of the leaves of basil plant showed that increasing in number against week for all treatments. This means by the encapsulation with PGPB was successful compared to the others. Encapsulation of PGPB showed the efficient of the plant growth and suitable for chemical fertilizer replacement. Lastly, further research is needed to justify the essential oil and chemical composition is influenced by the PGPR as the main idea for the next study.

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