Consortium Formulation of Bacteria as a Fertilizer and Biological Pesticide in Various Carriers

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Abstract. The use of agricultural production facilities that are produced from non-renewable natural resources such as fertilizers and chemical pesticides is constantly feared that will disrupt environmental sustainability and reduce soil fertility and quality. The application of biotechnology derived from local resources is a very appropriate alternative to answer these challenges, such as the use of local microorganisms that act as biological fertilizers and pesticides. The research aims to determine the compatibility of the bacterium Bacillus cereus strain ATCC 14579, Bacillus subtillis subsp. Subtilis strain 168 Bacillus siamensis strain KCTC13613, Azotobacter sp. and Pseudomonas fluorescens isolated from local microorganisms (MOL) banana weevil and know the best formulation, as well as the shelf life of the formula. The study was conducted in 3 stages: 1) testing bacterial compatibility on TSA media with the dual culture method; 2) test bacterial formulations on compost carriers, peat soils and vermicompost.3) Test the shelf life of formulas. The results showed that B. cereus strain ATCC 14579, B. subtillis subsp. subtilis strain 168, B. siamensis strain KCTC13613, P. fluorescens and Azotobacter sp were synergistic (compatible) so that they could be sponsored and formulated on carrier material (compost, kascing, peat). The best formulation is a formula for compost and peat soils added with Molas, CMC and Arginine. This formula has a shelf life of up to 6 months with the number of bacterial consortium colony of 4.64-4.67 x 10^5 CFU / gram, fulfilling the criteria of biological fertilizer according to Ministry of Agriculture Decree No. 261 KPTS SR 310 4, 2019 which is ≥ 105.

1. Introduction
The use of agricultural production facilities that are produced from non-renewable natural resources such as fertilizers and pesticides continuously with excessive amounts is feared to disrupt environmental sustainability. Awareness of the occurrence of negative impacts will cause reactions including the development of integrated pest control systems, changing the orientation of the agricultural system back to nature called organic farming.

The application of biotechnology derived from local resources is an alternative that is considered very appropriate to answer all these challenges, one of which is using local microorganisms (MOL) which have a multipurpose role so that they can be used as bio-fertilizers (bio-fertilizers) and bio-pesticides (biological pesticides). In previous studies Ref [1] isolated 5 types of bacteria from LMO base of banana stems namely Bacillus cereus strain ATCC 14579, Bacillus subtilis subsp. Subtilis strain 168 and Bacillus siamensis strain KCTC13613, Azotobacter sp. and Pseudomonas fluorescens. Bacteria B. cereus strain ATCC 14579, B. subtilis subsp. Subtilis strain 168, B. siamensis strain KCTC13613, P. fluorescens have the potential as biopesticides because they secrete extra cellular enzymes (chitinase,
These four bacteria can be used as bio-pesticides controlling blast in rice because they can inhibit the development of the fungus P. oryzae with a percentage of inhibition $\geq 75\%$. Azotobacter sp. less potential as a bio-pesticide controlling blast disease with a percentage of inhibition of 22% and a classification of weak inhibitory activity. B. cereus strain ATCC 14579, B. subtilis subsp. Subtilis strain 168 and B. siamensis strain KCTC13613, Azotobacter sp. and P. fluorescens have potential as bio-fertilizers because they can fix N from the air and as phospho-bacterin because they can dissolve phosphate [1].

The application of bacteria that acts as fertilizer and biological pesticides is generally still in the form of cell suspension, causing the bacterial population to decline rapidly so that it is not effective in controlling disease or increasing plant growth and production. This bacterium also competes with other microorganisms that have better adaptability. Therefore these bacteria need to be formulated so that population density can be maintained so that it is effective as a fertilizer and biological pesticide and makes it easier to use and market [2]. Carriers in the formulation include peat, clay, organic matter, tapioca flour and charcoal [3].

The formulation is mixing the organism in the carrier material, which is added with additional ingredients to maximize the ability to survive in storage, optimize the application of these microorganisms and protect them after application [4]. The purpose of making this formulation is to facilitate application in the field, transportation, and packaging, and can increase the effectiveness of the active ingredient used [5].

Bacterial formulation can be carried out with a single isolate or several bacteria that are consortification so that it has better potential than a single isolate. The consortium is a mixture of microbial populations in the form of communities that have cooperative, commensal and mutualistic relationships. Each member of the community will associate, so that it is more successful in degrading chemical compounds than a single isolate. Some of the bacteria that are consortified will not interfere with each other in sufficient substrate conditions [6]. Pseudomonas isolates, fluorescens 2 isolated from chrysanthemum ryzosphere plants in the Segunung area, and Bacillus subtilis obtained from IPB and Trichoderma harzianum obtained from UGM. are compatible with one another in media that contain lots of protein, like KingsB [7].

The use of a consortium consisting of a mixture of several bacteria has been reported. The consortium of bacteria formed naturally or artificially has a complementary metabolic function in an ecosystem [8]. Bacterial mixed cultures have a more perfect reshuffle ability and have a higher tolerance to toxic metabolites [9].

The formulation is the first step in the commercial development of biological fertilizers and pesticides. The principle of the formulation is to mix the organism in the carrier material, which is supplemented with additives to maximize the ability to survive in storage, optimize the application of target microorganisms and protect the organisms of fertilizer and biological pesticide after application [4].

The basic function of the formulation is to stabilize microorganisms during production, distribution and storage, change product applications, protect agents from environmental factors that can reduce the ability to survive and increase the activity of agents to control target organisms. The formulation consists of two types, namely solid-shaped products (flour and granules) and suspension (oil or water-based, and emulsions) [4].

The purpose of this study was to determine the compatibility of the 5 bacterial isolates tested to be consortified in one formulation and to find the right organic carrier to formulate the 5 bacteria, and to know the duration of the formula shelf life.

2. Materials and Method
The materials used in the study were isolates of B. cereus strain ATCC 14579, B. subtilis subsp. Subtilis strain 168 and B. siamensis strain KCTC13613, Azotobacter sp. and P. fluorescens media Tryptone Soy Agar (TSA), petridish, ose needle, cook borer with a diameter of 8 mm. Compost, molas, kascing, peat soil, arginine, CMC, King's B, NA.
2.1. Bacterial isolate compatibility test to be formulated.
Compatibility test of B. cereus strain ATCC 14579, B. subtilis subsp. Subtilis strain 168 and B. siamensis strain KCTC13613, Azotobacter sp. and P. fluorescens in vitro were performed using the dual culture method with Tryptone Soy Agar (TSA) media. The TSA media was heated until it thawed, after that, it was poured into a 15 ml/petridisc, then allowed to stand for 10 minutes until it was frozen. The isolates of each bacterium used for treatment were 2 days old. Each bacteria was tested for compatibility by etching the first isolate on TSA media. While the second bacterial isolate was inoculated by making a suspension well using a cook borer with a diameter of 8 mm. Isolates are stated to be compatible if there are no inhibition zones (clear zones) around the suspension well, and are stated to be incompatible if there are inhibition zones in the area where the two isolates meet the suspension wells [10].

2.2. Bacterial Formulation
Bacterial formulations on various carriers were carried out using a completely randomized design (CRD) with 6 treatments and 3 replications, so there were 18 experimental units. The bacteria formulated were a consortium of isolates of Bacillus cereus strain ATCC 14579, Bacillus subtilis subsp. Subtilis strain 168, Bacillus siamensis strain KCTC13613, Pseudomonas fluorescens and Azotobacter sp. With 6 formulas, they are:
A = Compost + Molas + 5 types of bacteria that are consortified
B = Compost + Molas + Arginine + CMC + 5 types of bacteria that are consortified
C = Peat soils + Molas + 5 types of bacteria that are consortified
D = Peat soils + Molas + Arginine + CMC + 5 types of bacteria that are consortified
E = Kascing + molas + 5 types of bacteria that are consortified
F. Kascing + Molas + Arginine + CMC + 5 types of bacteria that are consortified

3. Result and Discussion

3.1. Test results of bacterial isolate compatibility to be formulated
The results of observations of the bacterial compatibility test to be formulated are presented in Figure 1.

![Figure 1](image)

Caption:
a. B. siamensis compatibility with P. fluorescens
b. B. subtilis compatibility with Azotobacter sp
c. B. siamensis compatibility with B. substilis
d. Azotobacter sp compatibility with P. fluorescens
e. B. cereus compatibility with Azotobacter sp
f. B. siamensis compatibility with Azotobacter sp
g. B. cereus compatibility with B. substilis
h. B. siamensis compatibility with B. cereus
i. B. substilis compatibility with Azotobacter sp.

3.2. Bacterial formulation test results on various carriers
Population of bacterial consortium in several formulations aged 20 days after inoculation (DAI) is presented in Table 1.

| Treatment                                      | Bacterial population ( CFU/gram ) |
|------------------------------------------------|-----------------------------------|
| C= Peat soil + Molas                          | 8.24 x 10⁵ a                      |
| F= Kascing + Molas + CMC+Arginin              | 7.56 x10⁵ b                       |
| E= Kascing + molas                            | 5.53 x10⁵ c                       |
| D= Peat soil+ Molas + CMC + Arginin           | 5.28 x10⁵ cd                      |
| A= Compost + Molas                            | 5.22 x10⁵ d                       |
| B= Compost+ Molas + CMC+Arginin               | 4.84 x10⁵ e                       |
| Coef. of Variation (%)                       | 0.31                              |

Note. The numbers followed by the same letter and the same column are not significantly different in the Duncan α 0.05 test.

In Table 1 it can be seen that the bacterial formula of peat soil+ molas soil has the highest population of bacteria aged 20 DAI, followed by the treatment of Kascing + Molas + CMC + arginine + bacterial consortium, Kascing + Molas + bacterial consortium, and Compost + Molas + bacterial consortium, Compost + Molas + CMC + arginine + bacterial consortium, all six types of this formula have a significantly different bacterial population according to the 0.05 duncan test. Differentiation in the growth of each bacterium in formulation media from the first observation to the fifth observation can be seen in Figure 2.

![The growth of bacterial population in some formula](image)

**Figure 2.** The shelf life test results of the bacterial formulations were consortified.

The bacterial consortium population after being stored for 6 months in each formula can be seen in Table 2.
Table 2. Number of bacterial consortium colonies in 6 biological fertilizer and pesticide formulations 6 months after inoculation

| Treatment | Colony (CFU/gram) |
|-----------|-------------------|
| A= Compost + CMC + Arginin | 4.87 x 10^5 CFU a |
| C= Peat soil + CMC + Arginin | 4.64 x 10^5 CFU a |
| E= Kascing + CMC + Arginin | 2.68 x 10^5 CFU b |
| D= Peat soil + Molas | 1.64 x 10^5 CFU c |
| C= Kascing + Molas | 1.48 x 10^5 CFU c |
| A= Compost + Molas | 0.92 x 10^5 CFU d |

Coefficient of Variation (%) 4.2

Note. The numbers followed by the same letter and the same column are not significantly different in the Duncan α 0.05 test.

It is known that the formulation which has a high shelf life is the Compost and Peat formula added with Arginine and CMC (Table 2). It can be seen that the addition of CMC and L-Arginine is an amino acid with a high protein content so that it can increase the shelf life of fertilizer and biological pesticide formulas. Formulations with carbon sources only from molas cannot be stored longer, this can be seen from the growth of bacterial colonies in storage period for formulas from 1 to 6 months of age (Figure 3).

![Fig. 3. Growth of bacterial consortium colonies from 1 to 6 months after inoculation on each biological fertilizer and pesticide formula](image)

3.3. Analysis of the quality of biological fertilizers and pesticides

Analysis of the quality of biological fertilizer and pesticide formulations including nutrient content, C-organic and pH formulas conducted at the Central Plantation Service Laboratory, Pekanbaru (Riau).
Table 3. Results of nutrient analysis of 6 biological fertilizer and pesticide formulas 6 months after inoculation

| Treatment | pH  | N  | P  | K  | C- org. |
|-----------|-----|----|----|----|---------|
| F= Kascing+Molas+CMC+Arginin + Bacteria | 8.74 a | 0.72 | 0.65 | 0.31 | 32.7 |
| B= Compost+Molas+CMC+Arginin+ Bacteria | 8.7 a | 1.49 | 4.46 | 2.08 | 28.4 |
| D= Peat soil +Molas+CMC+Arginin + Bacteria | 8.59 a | 1.39 | 0.48 | 0.25 | 28 |
| E= Kascing + Molas +Bacteria | 7.42 b | 0.87 | 0.69 | 0.36 | 13.3 |
| C= Peat soil+ Molas+Bacteria | 6.32 c | 1.47 | 4.36 | 2.02 | 29.5 |
| A= Compost + Molas+Bacteria | 4.14 d | 1.21 | 0.29 | 0.4 | 29.4 |

Coef. of Variation (%) 6.8

Note: The numbers followed by the same letters in the same column are non-significantly different in the Duncan α 0.05 test

All combinations of bacterial isolates tested did not show any inhibition zones or clear zones around the bacterial colony wells (figure 1), According to Ref.[10]. Clear zones were formed due to competition in obtaining nutrients in the culture media by the isolates, so the population growth of one of the bacteria becomes faster than the other bacteria, which causes the food availability of the bacteria to be limited and its development is hindered then a clear zone is formed as a barrier zone due to the antagonistic nature of the bacterial isolate. The absence of this clear zone shows that B. cereus strain ATCC 14579, B. subtillis subsp. Subtilis strain 168 and B. siamensis strain KCTC13613, Azotobacter sp. and P. fluorescens are compatible so that they can be consortified and integrated into one formula of biological fertilizers and pesticides.

Bacterial consortium is a collection of bacteria that work together to form a community, to produce significant products [5],[11]. The compatibility or synergism of two or more inoculated bacteria is a very important factor so that the bacteria can work well together [12]. Bacteria with the same genus or species can interact and synergy, and share the same source of nutrition. This shows the cooperative behavior between bacteria in a habitat in the form of a consortium.

A consortium will produce the products that can be used together, so they can support the growth of a single isolate [13]. The mechanism of synergism between isolates in the consortium is still uncertainty, alleged several studies due to several factors, namely: [14]. one member of the genus is able to provide one or more nutritional factors that cannot be synthesized by other members of the genus, [15]. One member of the genus that is unable to degrade certain organic materials will depend on members of the genus who are able to provide the results of the degradation of the organic material,[16] one member of the genus protects members of other genera that are sensitive to the certain material of the organic substances by reducing the concentration of organic substances that are toxic by producing specific and non-specific protective factors [17].

The consortium test shows that there are complementary isolates working to degrade propoxur as a growth substrate [9]. Population of bacterial consortium on peat soil, vermicompost and compost in various formulas greater than 105 except compost formula added molas (Table 1), so that the formulation tested can be used as fertilizer and biological pesticides. This bacterial population has met the Indonesian national standard (SNI) of biological fertilizers. Based on the decision of the Minister of Agriculture No. 261 KPTS SR 310 4 2019 regarding the minimum technical requirements of organic fertilizer, biological fertilizer and soil enhancers for the bacteria Psedomonas, Bacillus and Azotobacter bacteria population is ≥ 105.

Bacterial population growth in the 6 formulas tested are fluctuated, after observation 1 (1 day after inoculation (DAI),) the bacterial population increased until the second observation (5 DAI), after that the bacterial population showed a decline on the third observation (10 DAI ) except the Compost + arginine + molas and Kascing + molas formulas, in these two formulas the bacterial population are still increasing until the third observation, after that it gradually decreases until the 5th observation (20 DAI).
However, the other 4 formulas after population decline at the third observation, the bacterial population increased again at the fourth observation and fell again at the 5th observation (20 DAI).

The growth of bacterial populations undergoes several phases in growth in the formulation medium (Figure 2). Bacterial population growth curves can be divided into several phases, namely lag phase, exponential phase (logarithmic), stationary phase and death phase [18]. The lag phase is the phase of cell physiological adaptation to maintenance conditions. The lag phase is used by microorganisms for cell enlargement, DNA synthesis, enzymes, and ribosomes. The exponential phase is characterized as a period of cell division when the rate of cell growth is proportional to the number of cells present at a given time. Microorganisms reach the maximum cell division rate during this phase. The exponential phase will continue as long as the cell gets enough nutrients and supported by environmental conditions [19].

The stationary phase is characterized by no increase or decrease in the number of cells. this is caused by the number of cells dividing in proportion to the number of dead cells. Cell death can be caused by the depletion of nutrients and oxygen. in addition, increasing in population density can cause the accumulation of organic acids and biochemical compounds that are toxic to cells. The next phase of the curve is the death phase. microorganisms die at a rapid rate, due to the depletion of nutrients and the formation of accumulated metabolic waste [20].

Formulations which have a high shelf life are the Compost and Peat formula added with Arginine and CMC (Table 2). It can be seen that the addition of CMC and L-Arginine is an amino acid with a high protein content so that it can increase the shelf life of biological fertilizer and pesticide formulas. CMC serves as an additional source of carbon is widely recommended in the product because it has no toxicity and has high solubility. While L-Arginine is a protector for bacteria in the formula. So that formulations with carbon sources only from molas cannot be stored longer, this can be seen from the growth of bacterial colonies in the formula storage period from 1 to 6 months of age (Figure 3).

Table 2 and Figure 3 show that the formulation using compost plus molas has the lowest number of bacterial consortium colonies and does not meet the criteria of biological fertilizer according to Decree of the Minister of Agriculture No. 261 KPTS SR 310 4, 2019 which is ≥ 105. While the other 5 formulas still meet the standards of biological fertilizers set by the Minister of Agriculture. Starting at 3 months the bacterial population in the formula Peat soils plus molas continues to decline until the age of 6 months of observation. The growth of bacterial populations in formulas is also influenced by the pH and nutrient content of the formula and C-organic (Table 3).

Bacterial population growth is strongly influenced by the pH and nutrient formulas. The compost plus molas formula has the lowest number of colonies after storage, this is due to the lowest pH of formula and significantly different compared to other formulas (Table 3), the pH of the formula for bacterial development is 7-8 [21].

The growth of bacterial colonies in the vermicompost + molas + arginine and CMC formulas was lower and significantly different from peat soils and compost because the nutrient N, P and K of the formula were very low despite having optimum C and pH. The element of N and P are the basic elements needed by bacteria for cell growth [22]. In the observation of the 5th bacterial colony (6 month shelf life) (Figure 2), it appears that all formulas tested except compost + molas showed a horizontal graph, it means that the additives in a formulation and the duration of storage affect the stability of the bacterial population, the formulation of a mixture of carriers (compost, vermicompost, peat soil) with Molas + CMC and L-arginine is quite good in supporting bacterial survival during storage [23].

4. Conclusion
From the results of the study it can be concluded that B. cereus strain ATCC 14579, B. subtilis subsp. Subtilis strain 168, B. siamensis strain KCTC13613, P. fluorescens and Azotobacter sp isolated from LMO of base of banana stems are mutually synergistic (compatible) so they can be conserved and formulated in the carrier (compost, vermicompost, peat soil). The best formulation is a formula with a mixture of compost and peat soils added with Molas, CMC and Arginine. This formula has a shelf life of up to 6 months. If the formulation is directly used or applied to the field to control pests and diseases and increase the growth of crop production, the formula of peat soil plus molas can be used because this
formula has a high number of bacterial colonies at the age of 20 days after inoculation. The cost of producing this formula is much cheaper and the carrier material is very easy to obtain.

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