The assay of carrier material and bacteria isolate formula as a biofertilizer on soybean in Inceptisols from West Java

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Abstract. Biofertilizer carrier material is one of the important factors in maintaining the viability and effectiveness of microbes when it is applied to soil and plant. This research aimed to assessed several carrier materials and isolate formulas for the growth and productivity of soybean. The experiment was conducted in greenhouse and arranged in a randomized completely block design with 2 factors and 3 replications. The first factor was the combination of the carrier material (peat, dolomite, zeolite, kaolin, biochar and rock phosphate) B1, B2, B3 until B12, while the second factor was the isolate formula, i.e.: I0 (Control), I1 (Rhizobium sp.+ Azotobacter sp.) and I2 (Rhizobium sp. + Azotobacter sp. + Bacillus sp.). The carrier material and microbial formula did not interact significantly with plant height, root dry weight and weight of filled pods. However, it gives significant interaction to the number of root nodules, plant dry weight, number of filled pods, soybean grain yield. The biofertilizer formulas of B3I1, B4I1 and B11I2 were consistent in increasing the number of filled pods and the grain weight of soybean compared to other formulas.

1. Introduction
Soybean productivity in Indonesia is still relatively low (1.57 t ha\(^{-1}\)) [1], it is not sufficient to meet domestic soybean needs. One of the efforts to increase soybean productivity is high yielding varieties [2]. The use of superior varieties must be supported by good soil management technology by liming, fertilizing with organic matter and using biological fertilizers [3-6]. The application of biofertilizer supported plant fertility, thereby increasing sustainable agricultural production.

Biofertilizer is a fertilizer containing beneficial microbes, both single and multiple microbes in one carrier material with the function of providing nutrients and increasing plant production. The microbes formulated are beneficial microbes and nonpathogenic for plants. Some of the microbes were used as biological fertilizers are from the symbiotic N\(_2\) fixing bacteria (Rhizobium sp.), nonsymbiotic N\(_2\) fixing bacteria (including Azotobacter sp. and Azospirillum sp.), P solubilizing microbes (Bacillus sp., Pseudomonas sp., Streptomyces sp. and the fungi Trichoderma sp., Aspergillus sp., Penicillium sp.) [7].

The use of biofertilizers requires appropriate carrier material. Carrier material serves to microbes’ growth, package and extend the shelf life of biological agents [8]. Carrier materials that can be used are peat, kaolin, dolomite, rock phosphate, biochar, vermicompost, lignite, compost and perlite. Several sources of the carrier material have certain characteristics. One of the sources is rock phosphate as a source of P, but it has a low solubility level, so it is necessary to add phosphate solubilizing bacteria to increase the solubility of P [9].
The formulation of the carrier material is very important to provide an environment for microbes to sustain life and improve the performance of microbes when applied. Incompatible carriers can reduce the functional microbial population. Therefore, it is necessary to evaluate the formulations of various carriers with suitable microbial isolates so that they can increase the effectiveness of biological fertilizers and have a longer shelf life to support soybean plant productivity. The research objective was to assessed several carrier materials and isolate formulas for the growth and productivity of soybean.

2. Materials and methods

2.1. Materials
The materials needed were several sources of carrier materials consisting of peat, dolomite, rock phosphate, kaolin and biochar. Meanwhile, the microbes used were *Azotobacter* sp., *Rhizobium* sp. and *Bacillus* sp. The indicator plant used was soybean Anjasmoro variety. The soil used in this experiment was Inceptisols taken from Cibungbulang Bogor, West Java. The soil was analyzed for Chemical and soil microbial content in Indonesian Soil Research Institute Laboratory, Bogor.

2.2. Greenhouse experiment
The study was carried out in the Indonesian Soil Research Institute greenhouse in Laladon Sindangbarang Bogor. The soil was taken at 0 to 20 cm depth and then air-dried. Furthermore, the soil is pounded and filtered using a 2 mm sieve. After that, the soil was put into pots as much as 5 kg each and given organic matter (cow manure 1 t ha$^{-1}$). Inorganic fertilizer rate was 50 kg ha$^{-1}$ Urea, 100 kg ha$^{-1}$ SP-36 and 100 kg ha$^{-1}$ KCl. The fertilizer rate was determined based on the results of soil tests using the “Upland Soil Test Kit” from Indonesian Soil Research Institute.

The treatment was arranged in a randomized completely block design with 2 factors and 3 replications. The first factor was the carrier material formula which consists of 12 formulas as shown in table 1. The second factor was 3 types of biological fertilizer inoculant formulations (I), namely I0 (control), I1 (*Rhizobium* sp. +*Bacillus* sp.) and I2 (*Azotobacter* sp.+*Rhizobium* sp.+*Bacillus* sp.). Two seeds of soybean were planted in each experimental pot and inoculated by a prototype of biofertilizer inoculant from each treatment.

| No | Formula Code | Composition |
|----|--------------|-------------|
| 1. | B1           | Peat+dolomite |
| 2. | B2           | Peat+dolomite+rock phosphate |
| 3. | B3           | Peat+kaolin |
| 4. | B4           | Peat+kaolin+rock phosphate |
| 5. | B5           | Peat+zeolite |
| 6. | B6           | Peat+zeolite+rock phosphate |
| 7. | B7           | Biochar+dolomite |
| 8. | B8           | Biochar+dolomite+rock phosphate |
| 9. | B9           | Biochar+kaolin |
| 10. | B10         | Biochar+kaolin+rock phosphate |
| 11. | B11         | Biochar+zeolite |
| 12. | B12         | Biochar+zeolite+rock phosphate |

Note: peat, dolomite, rock phosphate, kaolin and zeolite in the form of powder passes to the sieved of 30 to 60 mesh

The carrier materials are mixed evenly using a mixing device, then packed with heat-resistant plastic with a weight of 40 g each. Then all the packaged carriers sterilized at a temperature of 121 °C and a pressure of 1 atm for 15 minutes. Inoculation of bacteria into the carrier material formula that has been

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grown in alternative media was combined into 3 packages of bio-fertilizer microbial formulation, so that were 36 combinations of biological fertilizer packages and carrier material be obtained.

The parameters observed were chemical properties, soil microbial populations, plant growth, number of root nodules, dry weight of soybean plants, root dry weight, number of filled pods, the weight of filled pods, amount of soybean seeds and grain yield weight.

3. Results and discussion

3.1. Soil chemical and biological characteristics
Inceptisols in Bogor used in this research activity have a clay texture with a pH slightly acid close to neutral, low organic matter content and very low available N and P. The soil characteristics is shown in table 2.

| No. | Parameter | Value | Criteria |
|-----|-----------|-------|----------|
| 1.  | Soil texture: Sand (%) | 10 |  |
|     | Silt (%) | 39 | Clay |
|     | Clay (%) | 51 |  |
| 2.  | pH: H2O | 5.50 | Slightly acid |
|     | KCl | 4.80 | - |
| 3.  | Organic-C (%) | 1.14 | Low |
|     | Total N (%) | 0.13 | Very low |
|     | C/N | 9 | Medium |
| 4.  | Potential P (HCl 25%) | 102 |  |
|     | Available P (Olsen) (ppm) | 2.0 | Very low |
| 5.  | K-Morgan (ppm) | 623 | High |
| 6.  | Exchangeable cations: | 8.13 | Medium |
|     | Ca (cmolc kg⁻¹) | 6.40 | High |
|     | Mg (cmolc kg⁻¹) | 1.24 | Low |
|     | K (cmolc kg⁻¹) | 0.21 | Low |
|     | Na (cmolc kg⁻¹) | 15.78 | Low |
| 7.  | CEC (cmolc kg⁻¹) | 15.78 |  |
| 8.  | Base Saturation (%) | >100 | Very high |
| 9.  | Acidity: Al³⁺ | 0.00 | Very low |
| 10. | Soil microbial content (CFU g⁻¹): |  |  |
|     | P Solubilizing Bacteria | 2.0 x 10³ |  |
|     | Rhizobium sp. | 3.1 x 10³ |  |
|     | Azotobacter sp. | 3.7 x 10⁵ |  |

Source: [10]

The functional microbial population included P solubilizing bacteria (2.0x10³ cfu g⁻¹ soil), *Rhizobium* sp. (3.1 x 10³ cfu g⁻¹ soil), while the *Azotobacter* sp. (3.7 x 10⁵ cfu g⁻¹ soil), this shows that biological fertilizer inoculation is needed, because the amount of functional microbial population is still low.

3.2. Observation of plant growth
The combination of carrier material formula and the microbial formula did not interact significantly to the soybean plant height in the flowering phase (table 3).
Table 3. The effect of combination of carrier material formula and biofertilizer microbial consortia on plant height in the flowering phase of soybean.

| Carrier Materials       | I0           | I1           | I2           | Average       |
|-------------------------|--------------|--------------|--------------|---------------|
| B1 (Peat+dolomite)     | 100.67       | 101.58       | 96.75        | 99.67 a       |
| B2 (Peat+dolomite+rock phosphate) | 102.25       | 106.17       | 108.42       | 105.61 a      |
| B3 (Peat+kaolin)       | 112.08       | 101.50       | 82.83        | 98.80 a       |
| B4 (Peat+kaolin+rock phosphate) | 106.83       | 99.00        | 107.92       | 104.58 a      |
| B5 (Peat+zeolite)      | 96.83        | 111.00       | 105.83       | 104.55 a      |
| B6 (Peat+zeolite+rock phosphate) | 109.75       | 105.33       | 106.00       | 107.03 a      |
| B7 (Biochar+dolomite)  | 97.58        | 104.08       | 106.92       | 102.86 a      |
| B8 (Biochar+dolomite+rock phosphate) | 107.58       | 105.83       | 107.17       | 106.86 a      |
| B9 (Biochar+ kaolin)   | 90.58        | 106.42       | 82.92        | 93.31 a       |
| B10 (Biochar+kaolin+rock phospate) | 108.67       | 100.92       | 96.42        | 102.00 a      |
| B11 (Biochar+zeolite)  | 96.83        | 111.00       | 105.83       | 104.55 a      |
| B12 (Biochar+zeolite+rock phosphate) | 105.17       | 86.00        | 99.75        | 96.97 a       |

Average 103.63 a 102.59 a 100.98 a

CV (%): 9.96

Notes: Numbers followed by the same letter in rows and columns are not significantly different in 5% DMRT

*I0=control; I1= Rhizobium sp. + Bacillus sp.; I2 Azotobacter sp.+Rhizobium sp.+Bacillus sp.

Table 4. The effect of a combination of carrier material formula and biofertilizer microbial consortia on the number of root nodules of soybean plants in the flowering phase.

| Carrier material                   | I0           | I1           | I2           | Average       |
|------------------------------------|--------------|--------------|--------------|---------------|
| B1 (Peat+dolomite)                | 10.00 ab     | 22.50 d      | 15.50 bc     | 16.00         |
| B2 (Peat+dolomite+rock phosphate) | 15.50 bc     | 17.50 cd     | 11.00 ab     | 14.67         |
| B3 (Peat+kaolin)                  | 9.50 ab      | 28.00 e      | 20.00 cd     | 19.17         |
| B4 (Peat+kaolin+rock phosphate)   | 12.50 b      | 16.50 c      | 10.00 ab     | 13.00         |
| B5 (Peat+zeolite)                 | 17.50 d      | 20.50 cd     | 8.50 ab      | 15.50         |
| B6 (Peat+zeolite+rock phosphate)  | 7.00 a       | 21.00 d      | 17.50 cd     | 15.17         |
| B7 (Biochar+dolomite)             | 10.00 ab     | 13.50 bc     | 11.00 ab     | 11.50         |
| B8 (Biochar+dolomite+rock phosphate) | 16.50 c      | 8.50 ab      | 9.50 ab      | 11.50         |
| B9 (Biochar+ kaolin)              | 16.00 bc     | 14.00 bc     | 16.50 c      | 15.50         |
| B10 (Biochar+kaolin+rock phosphate) | 10.50 ab     | 15.00 bc     | 7.00 a       | 10.83         |
| B11 (Biochar+zeolite)             | 12.00 b      | 15.50 bc     | 13.00 b      | 13.50         |
| B12 (Biochar+zeolite+rock phosphate) | 7.50 a       | 11.00 ab     | 18.00 d      | 12.17         |

Average 12.04 16.96 13.13

CV (%): 6.48

Notes: Numbers followed by the same letter in columns are not significantly different in 5% DMRT

*I0=control; I1= Rhizobium sp. + Bacillus sp.; I2 Azotobacter sp.+Rhizobium sp.+Bacillus sp.

The results of statistical analysis on the combination of the type of carrier material formula and the microbial formula gave a significant difference on the number of nodules (table 4). The best biofertilizer formula is the B3I1 (Rhizobium sp.+Azotobacter sp. by using a carrier of peat+kaolin) produce the highest number of root nodules (28 nodules pot⁻¹) and was significantly different compared to other formulas, followed by B11I (Rhizobium sp.+Azotobacter sp. using a carrier of peat+dolomite) produces
root nodules of 22.50 nodules pot$^{-1}$. This indicates that the compatibility of the carrier material with the bio-inoculated microbes plays an important role in fixing nitrogen. Rhizobium inoculation can cooperate with soybean to increase the number of root nodules [11].

Table 5. The effect of combination of carrier material formula and biofertilizer microbial consortia on the dry weight of soybean plants in the flowering phase.

| Carrier material                  | Innoculants$^x$ | I0     | I1     | I2     | Average |
|-----------------------------------|-----------------|--------|--------|--------|---------|
| B1 (Peat+dolomite)               |                 | 16.45 ab | 15.90 ab | 18.70 bc | 17.02   |
| B2 (Peat+dolomite+rock phosphate)|                 | 18.10 bc | 17.60 bc | 17.70 bc | 17.80   |
| B3 (Peat+kaolin)                 |                 | 16.10 ab | 19.70 bc | 21.10 c  | 18.97   |
| B4 (Peat+kaolin+rock phosphate)  |                 | 14.45 a  | 14.05 a  | 15.65 ab | 14.72   |
| B5 (Peat+zeolite)                |                 | 18.90 bc | 18.15 bc | 15.40 ab | 17.48   |
| B6 (Peat+zeolite+rock phosphate) |                 | 20.45 bc | 20.35 c  | 16.40 ab | 19.07   |
| B7 (Biochar+dolomite)            |                 | 15.90 ab | 17.30 ab | 17.40 b  | 16.87   |
| B8 (Biochar+dolomite+rock phosphate) |           | 20.75 c  | 16.45 ab | 15.85 ab | 17.68   |
| B9 (Biochar+kaolin)              |                 | 17.40 b  | 19.50 bc | 16.00 ab | 17.63   |
| B10 (Biochar+kaolin+rock phosphate) |          | 18.30 bc | 18.70 bc | 18.50 bc | 18.50   |
| B11 (Biochar+zeolite)            |                 | 18.25 bc | 17.45 bc | 19.00 bc | 18.23   |
| B12 (Biochar+zeolite+rock phosphate) |             | 14.90 ab | 18.65 bc | 17.25 ab | 16.93   |
| **Average**                      |                 | 17.50   | 17.82   | 17.41   |         |

CV %: 2.09

Notes: Numbers followed by the same letter in columns are not significantly different in 5% DMRT

Table 6. Effect of combination of carrier material formula and biofertilizer microbial consortia on root dry weight of soybean plants in the flowering phase.

| Carrier material                  | Innoculants$^x$ | I0     | I1     | I2     | Average |
|-----------------------------------|-----------------|--------|--------|--------|---------|
| B1 (Peat+dolomite)               |                 | 2.05   | 2.25   | 3.55   | 2.62    |
| B2 (Peat+dolomite+rock phosphate)|                 | 3.20   | 3.10   | 3.45   | 3.25    |
| B3 (Peat+kaolin)                 |                 | 4.50   | 4.35   | 2.70   | 3.85    |
| B4 (Peat+kaolin+rock phosphate)  |                 | 2.85   | 4.15   | 3.50   | 3.50    |
| B5 (Peat+zeolite)                |                 | 1.95   | 3.90   | 2.90   | 2.92    |
| B6 (Peat+zeolite+rock phosphate) |                 | 2.00   | 3.75   | 3.00   | 2.92    |
| B7 (Biochar+dolomite)            |                 | 3.00   | 3.80   | 3.25   | 3.35    |
| B8 (Biochar+dolomite+rock phosphate) |           | 3.85   | 4.15   | 4.50   | 4.17    |
| B9 (Biochar+kaolin)              |                 | 4.25   | 3.00   | 3.95   | 3.73    |
| B10 (Biochar+kaolin+rock phosphate) |          | 3.50   | 4.25   | 3.95   | 3.90    |
| B11 (Biochar+zeolite)            |                 | 3.05   | 3.45   | 4.05   | 3.52    |
| B12 (Biochar+zeolite+rock phosphate) |             | 4.55   | 2.75   | 2.15   | 3.15    |
| **Average**                      |                 | 3.23 a | 3.58 b | 3.42 b |         |

CV %: 0.77

Notes: Numbers followed by the same letter in rows are not significantly different in 5% DMRT

$^x$I0=control; I1= Rhizobium sp. +Bacillus sp.; I2 Azotobacter sp.+Rhizobium sp.+Bacillus sp.
The carrier material formula and the microbial formula had a significant influence on the dry weight of soybean at the flowering phase (table 5). The biofertilizer formula B3I2 (Rhizobium sp+Azotobacter sp+Bacillus sp.) using a carrier of peat+kaolin gave the highest plant dry weight (21.10 g pot⁻¹), and was not significantly different from the B8I0 (Biochar+dolomite+rock phosphate without microbes) produce plant dry weight of 20.75 g pot⁻¹, the lowest dry weight of plants was B4I0 (14.45 g pot⁻¹). Carrier material formula that consist of biochar, dolomite and rock phosphate without inoculant can provide nutrient, dolomite to increased soil pH and rock phosphate to provide P to the soil. Islam Phosporus availability increased root improvement, stalk and stem vigor [11].

The combination of carrier material formula and the microbial formula was not significant effect on the dry weight of soybean roots (table 6). This shows that the increase in soybean plant dry weight is due to an increase in root dry weight. The type of carrier material formula and microbial inoculation showed a significant effect on root dry weight. The inoculation of I1 (Rhizobium sp. +Bacillus sp.) show the highest root dry weight. The inoculation of Rhizobium sp. effect on the N accumulation in soybean roots [12].

3.3 Observations during soybean harvest
The results of statistical analysis showed that there was a significant effect on the number of filled pods (table 7). The highest number of filled pods was in B4I1 (Rhizobium sp.+Bacillus sp. using a carrier of peat+kaolin+rock phosphate) the pods number was 53.

Table 7. Effect of combination of carrier material formula and biofertilizer microbial consortia on the number of filled pods.

| Carrier material | I0        | I1        | I2        | Average |
|------------------|-----------|-----------|-----------|---------|
| B1 (Peat+dolomite) | 48.33 cd  | 47.67 cd  | 48.00 cd  | 48.00   |
| B2 (Peat+dolomite+rock phosphate) | 49.33 d   | 46.67 c   | 48.00 cd  | 48.00   |
| B3 (Peat+kaolin) | 43.00 bc  | 51.67 de  | 48.00 cd  | 47.56   |
| B4 (Peat+kaolin+rock phosphate) | 44.67 bc  | 55.00 e   | 45.67 cd  | 48.44   |
| B5 (Peat+zeolite) | 48.67 cd  | 53.33 de  | 48.33 cd  | 50.11   |
| B6 (Peat+zeolite+rock phosphate) | 45.33 c   | 42.33 b   | 50.67 d   | 46.11   |
| B7 (Biochar+zeolite) | 46.67 c   | 35.67 a   | 48.33 cd  | 43.56   |
| B8 (Biochar+zeolite+rock phosphate) | 42.00 b   | 49.33 d   | 50.33 d   | 47.22   |
| B9 (Biochar+kaolin) | 48.33 cd  | 47.67 cd  | 46.67 c   | 47.56   |
| B10 (Biochar+kaolin+rock phosphate) | 45.67 c   | 47.00 cd  | 47.67 cd  | 46.78   |
| B11 (Biochar+zeolite) | 46.67 bc  | 41.33 b   | 51.67 de  | 46.56   |
| B12 (Biochar+zeolite+rock phosphate) | 47.33 cd  | 43.33 bc  | 48.00 cd  | 46.22   |
| Average         | 47.58     | 46.75     | 48.44     |         |

CV %: 6.42

Notes: Numbers followed by the same letter in columns are not significantly different in 5% DMRT

*I0=control; I1= Rhizobium sp. +Bacillus sp; I2 Azotobacter sp+Rhizobium sp.+Bacillus sp.
The carrier material formula and biofertilizer did not show a significant effect on the weight of filled pods (table 8). Statistical analysis show that carrier material formula and inoculants formula was not significantly different. I2 formula inoculation show the highest weight of filled pods and the formula of B8 (biochar+dolomite+rock phosphate) show the highest weight of filled pods. But, the combination of B8I2 was not show the highest number. The suitable combined application of inoculant to the carrier material more effective in increasing the weight of pods plant$^{1}$ [11].

Table 8. Effect of combination of carrier material formula and biofertilizer microbial consortia on the weight of filled pods.

| Carrier material                      | Innoculants$^\text{a}$  |
|--------------------------------------|--------------------------|
|                                      | I0            | I1            | I2            | Average      |
| B1 (Peat+dolomite)                  | 22.07         | 18.87         | 22.73         | 21.22 a      |
| B2 (Peat+dolomite+rock phosphate)   | 21.40         | 20.27         | 19.27         | 20.31 a      |
| B3 (Peat+kaolin)                    | 18.13         | 22.00         | 20.27         | 20.13 a      |
| B4 (Peat+kaolin+rock phosphate)     | 18.80         | 23.40         | 20.40         | 20.87 a      |
| B5 (Peat+zeolite)                   | 19.93         | 20.80         | 20.73         | 20.49 a      |
| B6 (Peat+zeolite+rock phosphate)    | 19.80         | 17.60         | 21.87         | 19.76 a      |
| B7 (Biochar+dolomite)               | 22.80         | 19.60         | 22.00         | 21.47 a      |
| B8 (Biochar+dolomite+rock phosphate)| 23.33         | 22.80         | 21.47         | 22.53 a      |
| B9 (Biochar+ kaolin)                | 20.33         | 19.40         | 22.53         | 20.76 a      |
| B10 (Biochar+kaolin+rock phosphate) | 19.60         | 20.20         | 21.27         | 20.36 a      |
| B11 (Biochar+zeolite)               | 21.07         | 18.73         | 23.87         | 21.22 a      |
| B12 (Biochar+zeolite+rock phosphate)| 20.13         | 20.93         | 19.93         | 20.33 a      |
| Average                              | 20.62 a       | 20.38 a       | 21.36 a       | 20.87 a      |

CV %: 2.60

Notes: Numbers followed by the same letter in rows and columns are not significantly different in 5% DMRT

Table 9. Effect of combination of carrier material formula and biofertilizer microbial consortia on the amount of soybean seeds.

| Carrier material                      | Innoculants$^\text{a}$  |
|--------------------------------------|--------------------------|
|                                      | I0            | I1            | I2            | Average      |
| B1 (Peat+dolomite)                  | 80.67 ab       | 73.33 a       | 97.33         | 83.78        |
| B2 (Peat+dolomite+rock phosphate)   | 86.67 ab       | 81.00 ab      | 76.00 ab      | 81.22        |
| B3 (Peat+kaolin)                    | 66.67 a        | 92.33 b       | 88.33 a       | 82.44        |
| B4 (Peat+kaolin+rock phosphate)     | 82.67 ab       | 93.00 b       | 83.33 ab      | 86.33        |
| B5 (Peat+zeolite)                   | 77.00 ab       | 83.33 ab      | 65.33 a       | 75.22        |
| B6 (Peat+zeolite+rock phosphate)    | 72.33 a        | 70.33 a       | 95.67 b       | 79.44        |
| B7 (Biochar+dolomite)               | 76.67 ab       | 72.67 a       | 73.00 a       | 74.11        |
| B8 (Biochar+dolomite+rock phosphate)| 85.00 ab       | 69.33 a       | 73.33 a       | 75.89        |
| B9 (Biochar+ kaolin)                | 82.67 ab       | 67.33 a       | 77.33 ab      | 75.78        |
| B10 (Biochar+kaolin+rock phosphate) | 74.33 ab       | 65.33 a       | 74.67 ab      | 71.44        |
| B11 (Biochar+zeolite)               | 76.67 ab       | 73.00 a       | 90.67 b       | 80.11        |
| B12 (Biochar+zeolite+rock phosphate)| 81.00 ab       | 74.67 ab      | 56.00 a       | 70.56        |
| Average                              | 78.53          | 76.3          | 79.25         | 78.53        |

CV %: 13.47

Notes: Numbers followed by the same letter in columns are not significantly different in 5% DMRT

$^1$I0=control; I1= Rhizobium sp. + Bacillus sp.; I2 Azotobacter sp.+Rhizobium sp.+Bacillus sp.
The combination of carrier material formula and the microbial formula showed a significant effect on the amount of soybean seeds (table 9). Formula B4I1 (Rhizobium sp.+ Bacillus sp. using a carrier of peat+kaolin+rock phosphate) produced the highest number of soybean seeds (93 grains pot\(^{-1}\)), the number of seeds was not significantly different from the number of seeds produced by treatment B3I1 (Rhizobium sp.+Bacillus sp. by using a carrier of peat+kaolin), B6I2 (Rhizobium sp.+Azotobacter sp.+Bacillus sp. using a carrier of peat+zeolite+rock phosphate) and B11I2 (Rhizobium sp.+Azotobacter sp. using a carrier of biochar+zeolite). From these results, good carriers for microbial formula I1 are peat+kaolin and peat+kaolin+rock phosphate. The suitable carrier material formula for microbial formula I2 was peat+zeolite+rock phosphate and biochar+zeolite.

A combination of carrier types formula and and microbial formulas showed significant effect on grain yield of soybean. The highest yield was achieved in B3I1 (Rhizobium sp.+Bacillus sp. using a carrier of peat+kaolin) the grain yield was 13.80 g pot\(^{-1}\), followed by B11I2 (Rhizobium sp.+Azotobacter sp.+Bacillus sp. using a carrier of biochar+zeolite) the grain yield of 13.60 g pot\(^{-1}\) and B4I1 (Rhizobium sp.+ Bacillus sp. using a carrier of peat+kaolin+rock phosphate) the grain yield was 13.33 g pot\(^{-1}\) (table 10). The increase of grain yield was in line with the increase in plant dry weight, number of filled pods and the number of soybean seeds. This indicates that the growth of plant dry weight parameter, the number of filled pods and the number of soybeans increases, this also affects the weight of the seeds produced (table 9).

From the observations, the formulas B3I1 (Rhizobium sp.+Bacillus sp. by using a carrier of peat+kaolin), B4I1 (Rhizobium sp.+Bacillus sp. using a carrier of peat+kaolin+rock phosphate) and B11I2 (Rhizobium sp.+Azotobacter sp.+Bacillus sp. using a carrier of biochar+zeolite) (table 10) were consistent in increasing the number of filled pods, the number of seeds and the weight of grain yield soybean. Increased nodulation, higher dry mater and grain yield production due to Rhizobium inoculation [11].

However, not all biofertilizer formulas have a positive effect on soybean grain yields (table 10). From the observations, the formulas B3I1 (Rhizobium sp.+ Bacillus sp. by using a carrier of peat+kaolin), B4I1 (Rhizobium sp.+ Bacillus sp. using a carrier of peat+kaolin+rock phosphate) and B11I2 (Rhizobium sp.+Azotobacter sp.+Bacillus sp. using a carrier of biochar+zeolite) were consistent in increasing the number of filled pods, the number of seeds and the weight of grain yield soybean. The proper type of carrier material is very important to maintain the amounts of inoculants and longer self-life. Chemical and physical characteristics of carrier material formula was different from each other, it can impact on the effectiveness of biofertilizer to support plant productivity [13].

### Table 10. Effect of combination of carrier material formula and biofertilizer microbial consortia on grain yield weight.

| Carrier material                              | Innoculants\(^{a}\) | I0       | I1       | I2       | Average |
|-----------------------------------------------|----------------------|----------|----------|----------|---------|
|                                               |                      | g        |          |          |         |
| B1 (Peat+dolomite)                            | 12.07 b              | 10.07 a  | 13.80 b  | 11.98    |         |
| B2 (Peat+dolomite+rock phosphate)             | 11.67 b              | 12.20 b  | 10.27 a  | 11.38    |         |
| B3 (Peat+kaolin)                              | 9.93 a               | 13.80 b  | 11.93 b  | 11.89    |         |
| B4 (Peat+kaolin+rock phosphate)               | 10.87 ab             | 13.33 b  | 12.47 b  | 12.22    |         |
| B5 (Peat+zeolite)                             | 11.40 ab             | 11.73 b  | 9.87 a   | 11.00    |         |
| B6 (Peat+zeolite+rock phosphate)              | 11.60 ab             | 10.33 a  | 13.33 b  | 11.75    |         |
| B7 (Biochar+dolomite)                         | 11.51 ab             | 10.92 ab | 10.99 ab | 11.14    |         |
| B8 (Biochar+dolomite+rock phosphate)          | 12.76 b              | 10.40 a  | 11.01 ab | 11.39    |         |
| B9 (Biochar+kaolin)                           | 12.41 b              | 10.11 a  | 11.61 ab | 11.38    |         |
| B10 (Biochar+kaolin+rock phosphate)           | 11.15 ab             | 9.81 a   | 11.25 ab | 10.74    |         |
| B11 (Biochar+zeolite)                         | 11.49 ab             | 10.94 ab | 13.60 b  | 12.01    |         |
| B12 (Biochar+zeolite+rock phosphate)          | 12.13 b              | 11.23 ab | 8.40 a   | 10.59    |         |
| **Average**                                   | **11.58**            | **11.24**| **11.54**|          |         |

Notes: Numbers followed by the same letter in columns are not significantly different in 5% DMRT

\(^{a}\)I0=control; I1= Rhizobium sp. +Bacillus sp.; I2 Azotobacter sp.+Rhizobium sp.+Bacillus sp.
4. Conclusions
The combination of various carrier material formulas and biofertilizer microbial consortia significantly effect on the number of root nodules, plant dry weight, number of filled pods and soybean grain yield. The biofertilizer formula of Rhizobium sp.+Azotobacter sp. using a carrier of either peat+kaolin or peat+kaolin+rock phosphate or biochar+zeolite was consistent in increasing the number of filled pods and the grain weight of soybean compared to other formulas. The selected carrier material should be easy to find (available), low cost and environmentally friendly.

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