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Growth and Yield Responses of *Zea mays* to Different Granule Biofertilizer Applications

Erny Ishartati[1,*], *Sufianto* Sufianto[1], *Aniek* Iriany[1], *Ali* Ikhwan[1], *Otto* Iskandar Kurniawan Putra[1], *Nguyen* Ngoc Huu[2], *Irum* Iqrar[3], and *Made* Jana Mejaya[4]

[1]Department of Agronomy, University of Muhammadiyah Malang, Jl. Raya Tlogomas No. 246, Malang 65144, East Java, Indonesia
[2]Department of Plant Biology, Faculty of Agriculture and Forestry, University of Tay Nguyen, 567 Le Duan St., 63100 Buon Ma Thuot City , Dak Lak Province, Vietnam
[3]Office of Research, Innovation and Commercialization (ORIC), The University of Lahore, 1-Km Defence Road, 54000 Lahore, Pakistan
[4]National Research and Innovation Agency (BRIN), Jl. M.H. Thamrin No 8, Special Region of Jakarta 10340, Indonesia

Abstract. One of several factors that could increase maize production is the use of biofertilizers produced from microorganisms. The objective of this study was to obtain a granule biofertilizer formulation with *Citrobacter murliniae, Raoultella terrigana,* and *Enterobacter hormaechei* bacteria in increasing the growth and yield of several maize cultivar. The research was conducted at the Integrated Laboratory of the Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang and experimental land in Singosari Malang, East Java, Indonesia from November 2020 to April 2021. The treatments were arranged in split plot design and repeated three times. The main plot was the density of Rhizobacteria which consisted of R_0 = without Rhizobacteria, R_1 = density 10^7, R_2 = density 10^8, and R_3 = density 10^9. Sub-plot was maize cultivar consisted of C_1: Pertiwi 3, C_2: Bisi 18, C_3: Bisi 2, C_4: Syngenta NK 6172, and C_5: Pioner P27. Each experimental unit was represented by five sample plants. Results of the study showed that the treatment of bacterial colony density on biofertilizer granules has not been able to increase the growth and yield of maize, except for the average weight of cob and weight of cob without maize on the various cultivar studied.

Keywords: Corn, .environmental friendly technology, maize, plant growth promoting rhizobacteria.

* Corresponding author: ishartati.erny@gmail.com

1 Introduction

Maize (*Zea mays* L.) is an important agricultural commodity that plays a strategic role in the development of agriculture and the Indonesian economy, thus making it included in the sustainable food self-sufficiency policy program. The development of this commodity, according to data from the Ministry of Agriculture, is more on the supply of industrial raw materials, especially the animal feed industry which reaches 55 %, while for food consumption it is around 30 %, and the rest is for other industrial needs and seeds [1, 2]. The use of maize for feed is more in demand by the feed industry because of its better nutritional content, high calories, and protein containing complete amino acids, besides that for livestock because the texture of maize is fresher than other feed ingredients animal feed production in 2020 grew by around 5 % to 6 % compared to the realization in 2019, with a volume reaching 21.53×10^6 t, up from the 2019 estimate which was set at 20.5×10^6 t [3–6]. With the increase in the animal feed industry, the demand for maize will also increase.

The challenge in sustainable maize self-sufficiency is to increase the quantity and quality of maize production. Several factors can increase production, one of which is the use of fertilizers. However, the continuous and unbalanced use of inorganic fertilizers results in damage to soil structure, compaction, decreased nutrient content, environmental pollution due to accumulation of chemical residues, and disruption of the growth of microorganisms in the soil [7–9]. Therefore, it is necessary to use nanotechnology biofertilizers produced from microorganisms of the genus Pseudomonas, Enterobacter, Azospirilum, Azotobacter, Burkholderia, Bacillus and Serratia which can cooperate with plants in nutrient absorption as a Plant Growth Promoting Rhizobacteria (PGPR) [10–14].

In sustainable agriculture, improvement of the efficiency of PGPR is used to reduce chemical fertilizer inputs needed for crop production. Plant growth Promoting Rhizobacteria (PGPR) acts as a colony in plant roots and promotes plant growth by producing and secreting various chemical regulators in the rhizosphere [15–18].

PGPR actively colonizes plant roots by having three main roles for plants, namely as a biofertilizer, biostimulant and bioprotectant. This grouping is based on the presence of certain functional groups that play a role in the tolerance mechanism. The grouping is based on the presence of certain functional groups that play a role in the mechanism of osmotic stress tolerance (osmoprotectants), synthesis of phytohormones and organopesticides. Metabolites that are osmoprotectants are identified from the presence of sugar functional groups and sugar alcohols (polyols), amino acids, and ammonium. Meanwhile, the metabolites thought to play a role in the synthesis of phytohormones were identified from the presence of indole and diterpenoid groups as precursors of indole acetic acid (auxin) and gibberellin. Finally, the metabolites suspected of acting as organopesticides were identified from the presence of organophosphate and organochlorine groups [19].

PGPR activities provide benefits for plant growth, either directly or indirectly. The direct effect of PGPR is based on its ability to provide and mobilize or facilitate the absorption of various nutrients in the soil as well as to synthesize and change the concentration of various growth-promoting phytohormones. While the indirect effect is related to the ability of PGPR to suppress pathogen activity by producing various compounds or metabolites such as antibiotics and siderophores. Microbial-based inoculants stimulate plant growth, enhance early root development and nutrient uptake resulting in increasing nutrient concentrations of corn. Microbial-based inoculants positively impact corn growth and nutrient concentration, especially during the late vegetative stages. The enhancement of nutrient concentrations (N, P, and K) was related to the capacity of microbial-based treatments to impact root morphology at early stages of corn growth [20–23].

Some PGPR inoculants have the potential to increase maize growth yield and nutrients uptake by significantly increasing plant height, shoot and seed dry weight, ear dry weight and length and number of seeds per row. Plants nutrient uptake of N, P, K, Fe, Zn, Mn, and Cu were also significantly influenced by application of PGPR(s) [24–26].

The objective of this study was to obtain a granule biofertilizer formulation consisting of bacterial colonies *Serratia marcescens* (Bizio 1823), *Serratia nematodiphila* (Zhang *et al.* 2009), *Enterobacter hormaechei* (Hoffman *et al.* 2016), *Enterobacter cancerogenous* [(Urošević 1966) Dickey & Zumoff 1988], *Enterobacter cloacae* [(Jordan 1890) Hormaeche & Edwards 1960], *Enterobacter asburiae* (Brenner *et al.* 1988), *Pseudomonas fluorescens* (Migula 1895) in increasing the growth and yield of several maize cultivar.

2 Materials and methods

The research was conducted at the Integrated Laboratory of the Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang and experimental land in Singosari, Malang, East Java (coordinates 7°54'45"S 112°38'01"E) with an altitude of 482 m asl, from November 2020 to April 2021. The results of the average chemical properties consisting of pH (1 : 5) H_2O is 5.9 (slightly acidic), N Kjedahl 0.57 (medium), P-available is 37.5 mg L^{-1} (very low), K-available is 25.79 mg L^{-1} (very low), C-organic by 1.79 % (low), CEC of 22.82 cmol (+) kg^{-1} (medium), BS is 57 % (medium). Analysis of chemical were carried out in the Soil, Plant, Fertilizer and Water Laboratory BALITSA – Indonesia.

The materials used in this study were five cultivars of maize [C_1: Pertiwi 3, C_2: Bisi 18, C_3: Bisi 2, C_4: Syngenta NK 6172, and C_5: Pioner P27], biofertilizer consist of seven bacterial isolates (*S. marcescens, S. nematodiphila, E. hormaechei, E. cancerogenous, E. cloacae, E. asburiae, P. fluorescens*), inorganic fertilizers, pesticides, and herbicides.

Field plots consisted of 20 rows with a distance of 0.75 m and a length of 5 m arranged in a split plot design with density of rhizobacteria colonies (DRC) which consists of R_0: without Rhizobacteria, R_1 : density 10^7, R_2 : density 10^8, and R_3: density 10^9 as the main factor and sub-plots are maize cultivar consisting of: C_1: Pertiwi 3, C_2: Bisi 18, C_3: Bisi 2, C_4: Syngenta NK 6172, and C_5: Pioneer P27. Each treatment was repeated three times so that in total there were 60 experimental units. Each experimental unit was represented by five sample plants. The biofertilizer granules were placed alongside the plant 3 wk and 7 wk after transplanting at a dose of 100 g $plant^{-1}$. Plant maintenance carried out includes the provision of inorganic fertilizers, irrigation and pest and disease control.

Variables observed included plant height, stem diameter, flowering age of male and female flowers, corn weight with cornhusk and without cornhusk, tip filling and weight of 100 kernels. Data were analyzed using Minitab 18 for Analysis of Variance (ANOVA), while difference between couples' treatment done using the Tukey HSD at the level of = 0.05.

3. Results and discussion

3.1 Plant growth

The results of the analysis of plant height and stem diameter showed no significant interaction between the DRC and maize cultivar. Separately, the DRC treatment was not significantly to plant height [Figure 1(a)] but was significantly different to cultivar treatment [Figure 1(b)]. Stem diameter character was significantly different only in DRC treatment [Figure 2(a)], but not in cultivar treatment [Figure 2(b)].

In Figure 1(a) it can be seen that the mean plant height was relatively the same at the DRC treatment, this indicates that DRC treatment did not have a significant effect on maize plant height. Figure 1(b) shows that the Pioneer P27 cultivar showed the highest average bias compared to another cultivar. Based on Figure 2(a) the average stem diameter in the DRC treatment in the DRC 10^9 treatment was greater than the other treatments, although it was not significantly different from the DRC 10^8 treatments. Figure 2(b) the cultivar wasn't significant to stem diameter.

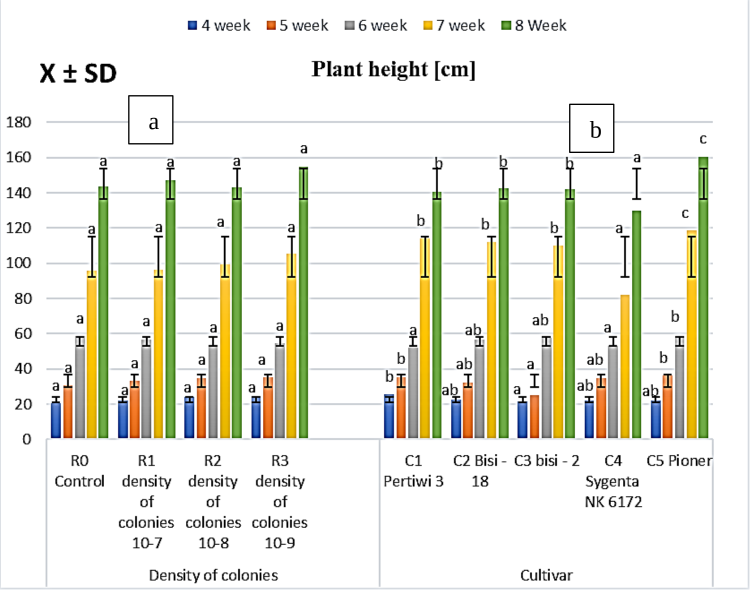

Fig. 1. (a) The average height of maize plants at DRC treatment, (b) the average height of maize at cultivar treatment.

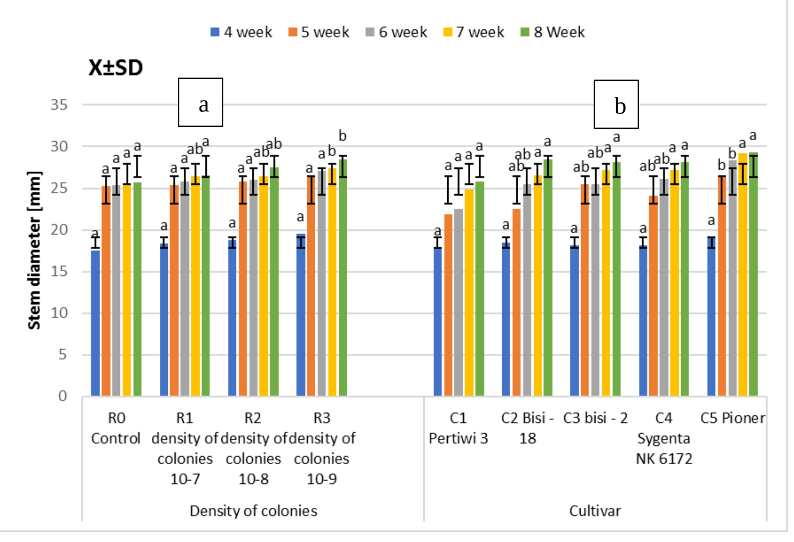

Fig. 2. (a) The average diameter of maize stem at DRC treatment, (b) the average diameter of maize stem at cultivar treatment.

The DRC treatment has a significant effect on stem diameter but did not affect plant height. DRC 10^8 and DRC 10^9 were treatments with the largest stem diameters. High DRC treatment can increase higher stem diameter. PGPR inoculated conditions measured thicker at the stem diameter [27].

In the cultivar treatment, Pioneer P27 showed the highest plant. This is presumably because the genetic expression of Pioneer P27 is different from another cultivar. Differences in plant height can be caused by differences in gene factors between cultivars. In general, the desired plant characteristics are short-stemmed plants. Smaller plants with strong stems and healthy growth are expected to reduce the risk of falling, reducing yields. Plants that are smaller making it easier for farmers to carry out the maintenance [28].

3.2 Flowering age characters

The results of the analysis of the flowering age male and female showed no significant interaction between the treatment of DRC and maize cultivar. Separately, the DRC was not significantly different to male and female flowers but was significantly different from the maize cultivar treatment (Figure 3).

In DRC treatment, the average of flowering age of male [Figure 3(a)] and female [Figure 3 (b)] showed the same mean. The results showed that DRC treatment has not affected its characters. In cultivar treatment, the average age of male [Figure 3(a)] and female flowering [Figure 3(b)] showed Bisi 18, and Pioneer P27 have a shorter flowering age compared with another cultivar, although they were not significantly different from Pertiwi 3. This indicates that cultivar Bisi 18 and Pioneer P27 are genetically shorter than other cultivars. The flowering age of the cultivar is between 54 d to 55 d after planting. This is the approach with the description of the company that produces it.

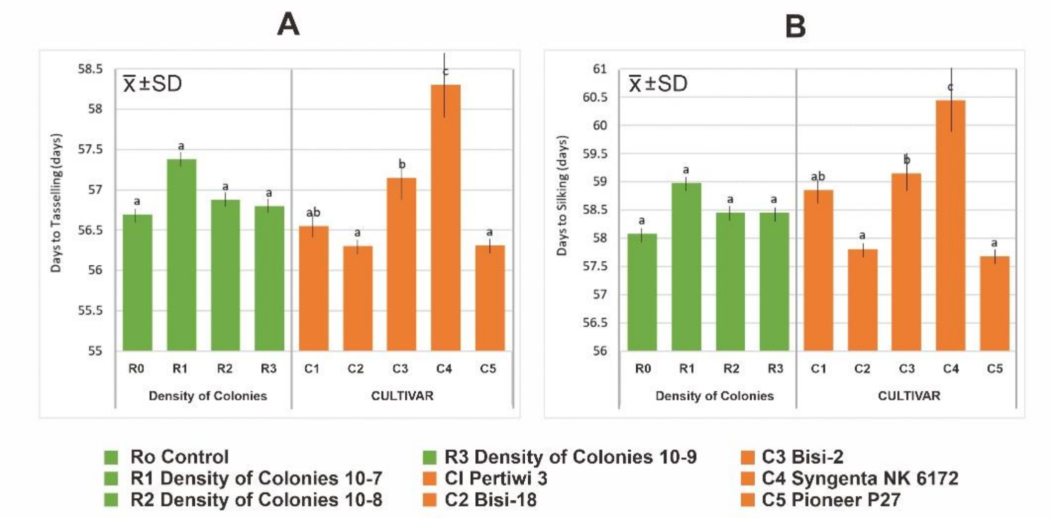

Fig. 3. (a) The average of flowering age male at DRC treatment and maize cultivar treatment, (b) the average of flowering age female at DRC treatment and maize cultivar treatment.

Flowering phases are an indicator of the transition from vegetative to generative phase. In the generative phase, the allocation of biomass to leaves and roots decreases while the allocation to stems increases with the phase of plant development [29]. The difference in the age of flower that occurs between cultivar is the genetic dominance in the cultivar. It has been shown that genetic, climatic, and other factors such as plant size affect the timing of flowering and its duration [30]. Maize yield is not only determined by kernel weight but also affected flowering time. Days to flowering recorded a positive and significant correlation with yield per plant. Because the productivity of yield is also determined by secondary plant characters, such as the Anthesis Silking Interval (ASI) character and the number or area of green leaves during flowering.

3.3 Yield characters

The results of the analysis of corn weight with cornhusk and without cornhusk (Figure 4) showed no significant interaction between the treatment of DRC and maize cultivar. Separately, the corn weight with cornhusk [Figure 4(a)] and without cornhusk [Figure 4(b)] on the DRC treatment and the maize cultivar treatment were significantly different.

Based on Figure 4(a) average corn weight with cornhusk and Figure 4(b) average corn weight without cornhusk on DRC treatment, DRC 10^9 was higher than the other treatments, although it was not significantly different with DRC 10^7 and DRC 10^8 (Figure 4). The addition of biofertilizers consisting of bacteria that have the ability to produce phytohormones positively can increase the weight of corn. Application of N-fixing bacteria and P-solubilizing bacteria on maize cultivation increased root and shoot weight and maize yield [31].

In the cultivar treatment, Pertiwi 3 showed the highest weight with cornhusk, although the bead was not significantly different from Pioneer P27 [Figure 4(a)]. This shows that the cultivar Pertiwi 3 and Pioneer P27 cultivar are genetically heavier than other cultivars. Following the description of the company that produces it, Pertiwi 3 has a potential harvest of 13.74 t ha^{-1} and Pioneer P27 12.1 t ha^{-1}. In the cultivar treatment, Pertiwi 3 showed the highest cob weight, although the bead was not significantly different from Pioneer P27 [Figure 4(b)]. These results aren't different from the weight of cornhusk. This shows that even though the corn has been separated from the husk, it still has a high weight compared to other cultivar in terms of differences produced by the genetics of the cultivar. Genetic factors will play a good role if environmental factors are in optimum condition or if environmental factors are in optimal condition, plant growth and yield will be largely determined by genetic factors [32].

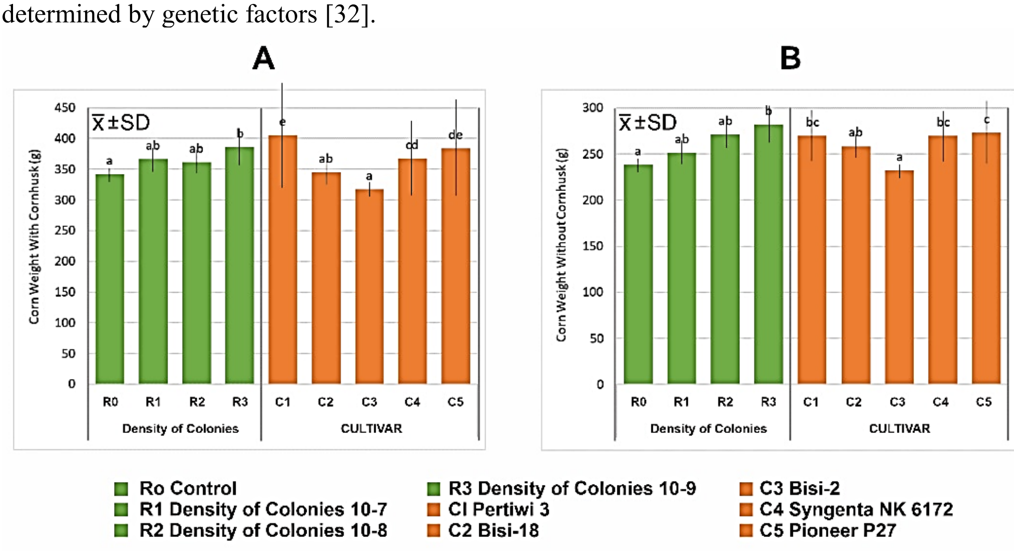

Fig. 4. (a) The average corn weight with cornhusk on DRC treatment and maize cultivar, (b) the average corn weight without cornhusk DRC treatment and maize cultivar.

The results of the analysis of the average weight of 100 grains and the average tip filling showed no significant interaction in the treatment of bacterial colony density and maize cultivar. Separately, the average weight of 100 grains in the bacterial colony density treatment showed no significant difference but in the cultivar treatment it was significantly different [Figure 5(a)], while the average tip filling in the bacterial colony density treatment showed no significant difference but in the cultivar treatment it was significantly different [Figure 5(b)].

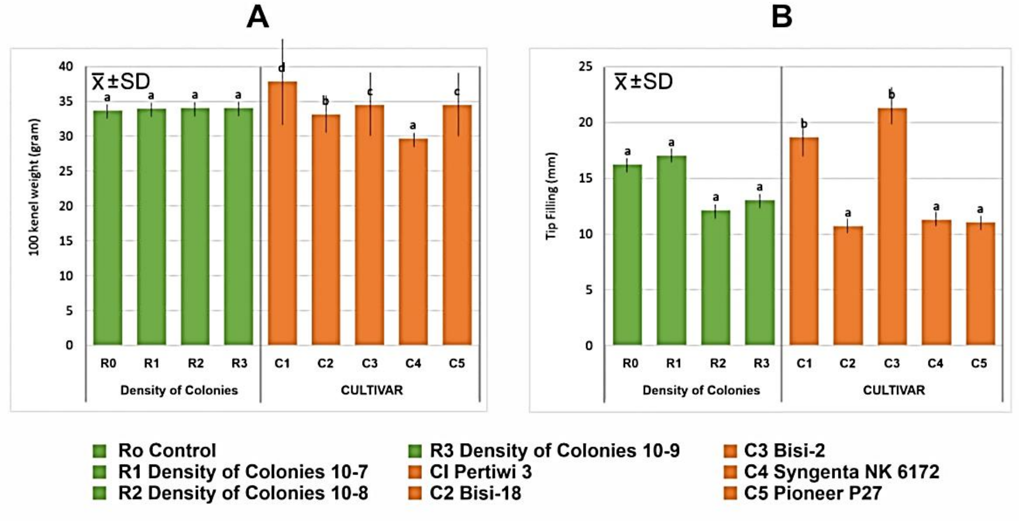

Fig. 5. (a) The average weight of 100 seeds in the bacterial colony density treatment and maize cultivar, (b) average tip filling in bacterial colony density treatment and maize cultivar.

In DRC treatment, the average of 100 kernel weight [Figure 5(a)] and tip filling [Figure 5(b)] showed the same mean. The cultivar wasn't significant to weight of 100 kernel and tip filling. 100 kernel weight was significantly affected due to cultivar treatments, Pertiwi 3 showed a significant difference in the weight of the highest 100 kernels [Figure 5(a)]. Differences in kernel weight can be caused by differences in gene factors between cultivars. The weight of 100 kernels is indeed heavier than the description of the cultivar because the weight of 100 kernels here is compared to the wet weight. Kernel weight is dependent on several factors, are the number of endosperm cells and starch granules that are determined in the lag phase of kernel growth during the first two weeks after pollination [33].

As for the tip filling variable, the largest value was found in the Bisi 2 cultivar, although it was not significantly different from the Pertiwi 3 cultivar [Figure 5(b)]. The value of the filling tip of each cultivar varies greatly. This is influenced by the process of plant pollination [34].

The tip filling of maize is largely determined by the filling of seeds on the cobs. The desired cob yields are those that have a low tip filling value. The smaller the tip filling value, the more filled the seeds on the cob. Thus, the desired percentage of grain filling on the cobs is 100 %.

3.4 Future research

This research was carried out on land with low fertility [35, 7], as shown in Chapter 2. In future research, the application of PGPR should be combined with organic fertilizer. Several researchers [36–38, 7– 9] stated that soil organic matter content will increase and can be used as an energy source for the growth maintenance of microorganism cells and the production of extracellular enzymes. Components of cellulose and lignocellulosic organic matter are broken down into simple carbohydrates such as glucose for growth. Increased glucose levels cause increased bacterial growth. However, the use of compost requires caution. Compost can increase soil fertility, but this organic fertilizer is one of the spreaders of microplastic contamination [39–41].

4 Conclusion

In this study, granules DRC treatment have not been able to increase the growth and yield of maize, except for the average stem diameter, weight with corn husk, and weight without corn husk maize on the various cultivars studied. Nonetheless, the rhizobacteria (S. *marcescens, S. nematodiphila, E. hormaechei, E. cancerogenous, E. cloacae, E. asburiae, P. fluorescens*) have the potential to be developed and can be applied to different plants and with different densities.

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