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Original Research Article

Effect of seed soaking with bacillus sp and organic fertilizer on growth of mustard green (*Brassica juncea* L.)

I Ketut Widnyana*, I Wayan Seputra Kuspianta, Putu Lasmi Yulianthi Sapanca
Faculty of Agriculture, Mahasaraswati University of Denpasar, Bali, Indonesia

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**Corresponding author email:*
widnyanaketut@gmail.com

Abstract

Bacillus sp. is one type of bacteria that functions as PGPR (Plant Growth Promoting Rhizobacteria). This study is designed to determine the effect of treatment duration time immersion of mustard seeds with Bacillus sp and the doses of organic fertilizer on growth and yield of mustard plants. The research was designed using Randomized Block Design (RBD) Factorial with two treatments i.e. time soaking of mustard seed with Bacillus sp. and organic fertilizer dose. The results showed that there was a very significant interaction (P <0.01) between the treatment of soil seed immersion with Bacillus sp. and the dosage of organic fertilizer on most parameters observed, except for the root length of the plant. The highest fresh weight of the mustard plant was found in the combination of the treatment of soaking of mustard seeds with Bacillus sp. for 20 minutes with a dosage of 1.50 kg organic fertilizer per plant that is 131,900 g, and the lowest was found in the combination treatment of soaking of mustard seeds for 30 minutes with a dosage of 0 kg organic fertilizer i.e. 34.333 g, so an increase of 384.178%. The highest drying weight of the mustard oven was found in a combination of treatment of soaking of mustard seeds with Bacillus sp. for 30 minutes with a dosage of 1.50 kg organic fertilizer per plant of 8,000 g, and the lowest was found in a combination treatment of immersion of mustard seed for 10 minutes with a dose of organic 0 kg fertilizer per plant that is 4.617 g.

Keywords: Soaking of Seed, Bacillus sp., PGPR, Organic Fertilizer

Introduction

Mustard green (Brassica juncea L.) called sawi hijau (Indonesia); sawi bunga (Malaysia); phakkwangtung or phakkatkheokwangtung (Thailand); False pakchoi, Mock pakchoi (English); Cai xin (China) is a vegetable that is quite popular, easily cultivated and can be eaten fresh (usually withered with hot water) or processed into pickles. Mustard green has a wide environmental adaptation, both in the highlands and in the lowlands and widely consumed by people, short-lived 20-45 days. When these vegetables are left to age 60-65 days after planting, it will usually be harvested seeds (Poespodarsono, 1998).

Mustard green is very important role for the health of the human body because these vegetables contain vitamins, minerals and vegetable proteins that are needed to improve the nutritional value of food. Each adult is estimated to require 150 grams of vegetables daily (Sunaryono, 1994). In addition, the development of tourism sector in the area of Bali provides development opportunities for the agricultural sector, especially vegetable cultivation for there are 33 types of vegetables needed by hotels and restaurants. Some

vegetables are still imported from outside the island (Antara and Susrusa, 1991).

The last decades of mustard green planting completely use chemical fertilizers as well as synthetic chemical pesticides. This type of cultivation is not good for consumer health, soil ecosystem damage physically, chemically and biologically. Technical improvements of mustard green cultivation should be pursued by utilizing environmentally friendly local resources, such as the utilization of PGPR bacteria such as Bacillus sp. derived from bamboo rhizosphere and combined with organic fertilizers sourced from the surrounding environment such as crop wastes, cattle dung which are abundant but not well utilized.

Rhizobacteria is a group of bacteria with plant roots habitat area (rhizosphere) that has been researched and proven to improve soil fertility, increase plant resistance and can suppress plant pathogens. Rhizobacteria act directly as biological fertilizer and stimulants biologically by producing hormones to grow crops such as IAA (indole acetic acid), gibberellin, cytokine, ethylene, dissolving minerals, and indirectly also prevents pathogenic microorganisms through formation of siderophore and an antibiotic (McMilan, 2007 ; Sarma et al., 2009). Bacillus sp. is a gram-positive bacteria are often used as the biological control of the root disease. The results have shown the potential of using selected strains of B. subtilis in the biological control of seed pathogens, as well as in promoting soybean growth (Araújo et al., 2005).

Bacillus sp. produce phytohormones which is useful directly or indirectly in the farm system. The benefits of phytohormones directly are able to increase the growth of plants and act as facilitators in nutrient uptake, and indirectly as an inhibitor of pathogenic activity in plants (Dwipayana, 2010).

Several studies have been done related to the utilization of PGPR in the cultivation of plants. Several researchers proved that treatments with Pseudomonas spp. can improve the plant growth of wheat and protect them from infection of Phytium spp. through seed treatment (Weller and Cook, 1986). Pseudomonas spp. can increase the number of leaves, height, and yield of tomato plants up to 81,7% through soaking of tomato seeds in suspension of the bacteria (Widnyana et al., 2013). Bacillus sp. tends to give a tomato seedling growing faster than the Pseudomonas spp. isolates until 2-3 days, while a mixture of both isolate the bacteria does not give a different impression with a single treatment (Widnyana and Javandira, 2015).

The study is conducted to obtain information on the benefits of Bacillus sp. rizobacteria combined with organic fertilizer for the growth of mustard green plants in the hope of suppressing the use of synthetic chemical fertilizers.

Material and Methods

The research was conducted during August and September 2016 in a greenhouse in Badung regency Bali Indonesia. The materials used are green mustard seed, PGPR Bacillus sp. suspension from bamboo rizosphere, organic fertilizer, and sterile soil. The research was designed using Factorial Random Block Design consisting of two factors: the time of soaking of mustard green seed with Bacillus sp. (T) and the dose of organic fertilizer (D). There are 3 levels of immersion time that is 10 (T1), 20 (T2), and 30 minutes (T3), with 4 levels of fertilizer dosage : without organic fertilizer (D0), 0.50 kg organic plant^{-1} (D1), 1.00 kg organic plant^{-1} (D2) and 1.50 kg organic plant^{-1} (D3). Thus there are 12 combinations of treatments with 3 replications so that there are 36 experimental units.

Green mustard seeds were soaked with Bacillus sp. suspension with a concentration of 10 ml in 1 liter of sterile water according to the treatment. Furthermore in the seedling nursery media. After the green mustard seedlings 4 leaves (age 14 days) are transferred to polybags that already contain soil mixed with organic fertilizer (2: 1) that has been roasted to minimize contaminants. Harvesting is done at the time of the growth of mustard green 21 days that has been marked by the emergence of flowers in most plants.

Results and Discussion

The results showed the significant interaction effect ($P < 0.05$) to very significant ($P < 0.01$) between PGPR Bacillus sp. soaking time with a dose of organic fertilizer on parameters plant height, number of leaves, plant fresh weight, root fresh weight, oven dry weight, and fresh weight of green mustard greens ha^{-1}; but did not interact significantly ($P > 0.01$) to the roots length of mustard green. Effect of soaking time interaction seed treatment with Bacillus sp. and dose of organic fertilizer to green mustard plants are presented in Table 1.

Table 1. Effect of interaction between time of soaking with Bacillus sp. and organic fertilizer dosage to mustard green height (cm).

	Plant height (cm)			
Treatment	Dose of organic fertilizer (D) (kg plant^{-1})			
	0 (D_0)	0,50 (D_1)	1,00 (D_2)	1,50 (D_3)
Soaking seed (T)				
10 minute (T_1)	29,633 fg	40,400 b	46,167 a	44,167 a
20 minute (T_2)	29,733 ef	42,933 b	44,000 ab	46,233 a
30 minute (T_3)	27,033 g	35,033 d	40.400 c	40,967 b

Note: values on rows and columns followed by the same letter, are not significantly different in DMRT 5%

The result of statistical analysis in Table 1. Shows that the combination of T2D3 treatment reached maximum plant height of 46,233 cm and not significantly different with T2D2, T1D3 and T1D2 treatment. Whereas minimum plant height reached at 27.033 sm in T3D0. This suggests that soaking mustard greens for 10 minutes or 20 minutes combined with a 1 kg or 1.5 kg fertilizer dose gives the same effect to the height of mustard green plants.

Effect of interaction soaking time seed treatment with Bacillus sp. and organic fertilizers on the number of green mustard leaves are showed in Table 2.

Table 2. Effect of interaction between the time soaking seeds with Bacillus sp. and a dose of organic fertilizer to the number of green cabbage leaves (leaf).

	Number of leaves (leaf)			
Treatment	Dose of organic fertilizer (D) (kg plant^{-1})			
	0 (D_0)	0,50 (D_1)	1,00 (D_2)	1,50 (D_3)
Soaking seed (T)				
10 minute (T_1)	6.933 cd	7,067 b	8,267 a	7,800 a
20 minute (T_2)	6,067 d	7,267 a	7,333 a	7,533 a
30 minute (T_3)	7,467 a	8,000 a	7,800 a	8,133 a

Note: values on rows and columns followed by the same letter, are not significantly different in DMRT 5%

The result of statistical analysis in Table 2. Showed that the highest number of leaves was found in T3D3 with mean of 8,133 strands, but not significantly different with T3D2, T3D1, T3DO, T2D3, T2D2, T2D1, T1D3 and T1D2 treatment. The lowest number of mustard green leaves found in T2D0 is 6,067 and not different with T1D0. These results indicate that the doses of organic fertilizers give almost the same effect to the number of mustard green leaves as long as the green mustard seeds are soaked 20 to 30 minutes.

In the root mustard green parameter, there was no interaction between the treatment of soaking time with Bacillus sp. and the dosage of organic fertilizer, so that the result of the research was analyzed to obtain the effect of single factor, as presented in Table 3.

Table 3. Effect of soaking treatment of mustard green seed with Bacillus sp. and dosage of organic fertilizer on root length of plant^{-1} (cm).

Treatment	root length of mustard green (cm)
Soaking seed (T)	
10 minute (T_1)	10,19 c
20 minute (T_2)	13,01 b
30 minute (T_3)	15,01 a
BNT 5%	0,087
Dose of organic fertilizer (D) (kg plant^{-1})	
0 kg (D_0)	11,29 a
0,50 kg (D_1)	12,50 a
1,00 kg (D_2)	13,52 a
1,50 kg (D_3)	13,64 a
BNT 5%	2,60

Note: The values followed by the same letter on the same treatment and column are not significantly different on LSD 5%.

The statistical analysis of the effect of the treatment on length of root mustard as presented in Table 3 shows that soaking with Bacillus sp. for 30 minutes gives the best effect to the root of the green mustard with an average of 15.01 cm, in contrast with 20 minutes (13.01 cm) and soaking 10 minutes (10.19 cm). But the treatment of organic fertilizers with different doses did not give a real different effect on root mustard green length.

The effect of the interaction of time soaking of the mustard seed with Bacillus sp. and the dosage of

organic fertilizer to the fresh weight of the mustard green plant is presented in Table 4.

Table 4. Effect of the interaction between green mustard seed soaking treatment with Bacillus sp. and a dose of organic fertilizer to the plant fresh weight (g).

	The fresh weight of the plant $^{-1}$ (g)			
Treatment	Dose of organic fertilizer (D) (kg plant^{-1})			
	0 (D_0)	0,50 (D_1)	1,00 (D_2)	1,50 (D_3)
Soaking seed (T)				
10 minute (T_1)	36,333 ef	63,333 cd	96,000 a	104,000 a
20 minute (T_2)	27,667 f	74,000 bc	85,333 abc	109,333 a
30 minute (T_3)	29,667 f	62,667 d	87,000 abc	104,000 a

Note: values on rows and columns followed by the same letter, are not significantly different in DMRT 5%

The result of statistical analysis of parameter to fresh mustard green weight in Table 4, indicated that the highest fresh green mustard weight found in T2D3 is 109.333 g is not significantly different from T1D3, T3D3, T2D2 and T3D2 treatment. The lowest fresh mustard green weight found in T2D0 treatment of 27,667 g was not significantly different from T1DO and T1D3. These results indicate that the soaking time of 10, 20, and 30 minutes combined with a dose of 1 kg or 1.5 kg per plant gives no significant effect on the mean fresh weight of the green mustard.

The effect of the interaction of soaking mustard seeds with Bacillus sp. and the dosage of organic fertilizer to oven dry weight of mustard green plant is presented in Table 5.

The statistical analysis of dry oven mustard green weight in Table 5 shows that the highest dry mustard green oven weight found in a combination of T3D3 treatment of 8,000 g was not significantly different from T3D2, T3 D1, T3D2, T2D2 and T1D2. The lowest dry oven mustard green weight found in T1D0 treatment of 4,100 g was significantly different from all other treatments except T1D3, T2D3, and T3D3.

The effect of the treatment of soaking time of the mustard seed with Bacillus sp. and the dosage of organic fertilizer to the fresh weight of the mustard green ha^{-1} is presented in Table 6.

Table 5. Effect of interaction between soaking treatment of mustard green seed with Bacillus sp. and dose of organic fertilizer on dry weight of oven crop^{-1} (g).

	Oven dry weight of plant^{-1} (g)			
Treatment	Dose of organic fertilizer (D) (kg plant^{-1})			
	0 (D_0)	0,50 (D_1)	1,00 (D_2)	1,50 (D_3)
Soaking seed (T)				
10 minute (T_1)	4,100 c	4,617 c	6,100 abc	7,347 ab
20 minute (T_2)	4,533 c	5,310 bc	5,867 abc	7,833 ab
30 minute (T_3)	4,617 c	5,333 bc	6,367 abc	8,000 a

Note: values on rows and columns followed by the same letter, are not significantly different in DMRT 5%

Table 6. Effect of interaction between soaking time treatment of mustard green seed with Bacillus sp. and dose of organic fertilizer on fresh weight of mustard green ha^{-1} (t)

	Plant fresh weight ha^{-1} (t)			
Treatment	Dose of organic fertilizer (D) (kg plant^{-1})			
	0 (D_0)	0,50 (D_1)	1,00 (D_2)	1,50 (D_3)
Soaking seed (T)				
10 minute (T_1)	9,184 cd	21,088 ab	23,980 a	23,810 a
20 minute (T_2)	8,163 d	24,490 a	24,150 a	25,850 a
30 minute (T_3)	7,313 d	17,007 b	23,469 a	21,088 ab

Note: values on rows and columns followed by the same letter, are not significantly different in DMRT 5%

The result of statistical analysis on the fresh weight of mustard green ha^{-1} shows that after conversion in the hectare planting area (ha^{-1}) the highest production is found in T2D3 treatment (soaking seed 20 minutes and 1.5 kg of organic fertilizer per plant) of 25,850 tons ha^{-1}. This result is the highest yield potential of the type of green mustard Tosakan variety in accordance with the product description of 20 - 25 tons ha^{-1}. Production of green mustard on T2D3 is not different from T2D1

(24,490 ton ha^{-1}), T2D2 (24,150 ton ha^{-1}), T1D2 (23,980 ton ha^{-1}), T1D3 (23,810 ton ha^{-1}), T3D3 (21,088 ton ha^{-1}) and T1D1 (21,088 tons ha^{-1}).The lowest production of mustard green was found in a combination of T3D0 treatment of 7,313 tons ha^{-1} was not significantly different from T2D0 (8,163 ton ha^{-1}) and T1D0 (9,184 ton ha^{1}).

Based on the understanding of previously presented data it can be stated generally that the immersion of green mustard seed with Bacillus sp. PGPR rizobakteri for 10, 20 and 30 minutes combined with 1 kg organic fertilizer dose or 1.5 kg per plant gives no significant effect to plant growth parameters such as plant height, leaf number, fresh weight of plant, oven dry weight of plant and fresh weight of mustard green ha^{-1}. Plant growth promoting bacteri (PGPB) are naturally soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion. This is in line with opinion Antoun and Kloepper (2001), inoculation of crop plants with certain of PGPB at an early stage of development improves biomass production through direct effects on root and shoot growth. The results of this study according to opinion Widnyana and Javandira (2015) that tomato seed treatment by soaking period of Bacillus sp. isolates between 10 to 30 minutes does not give a different effect on the time of germination and growth of tomato seedlings. Similar research that has been done by Widnyana, et al. (2018) showed that the soaking treatment of Swamp Cabbage (Ipomoea reptans Poir) seeds with suspense P. alcaligenes as PGPR encouraged 25% faster germination, increased yield by 24.4%, increased leaf count to 23.15%, extended stem until 25%, extend the roots to 46.90%, and increase the weight of fresh stems up to 67.07% and dry oven bar stems up to 84.21% compared to soaking the seeds in sterile water.

Rhizobacteria Bacillus sp. can produce phytohormones that have the potential to develop sustainable agricultural systems. The phytohormones produced by these soil bacteria can affect plant growth, either directly or indirectly. The phytohormone indirectly inhibits pathogenic activity in plants, whereas the immediate effect of fitohormon is to increase plant growth and can act as a facilitator in the absorption of some nutrients from the environment.

However, if considered in terms of efficiency of organic fertilizer use and based on the extent of planting ha-1 it is important to know that to obtain maximum potential yield with the lowest use of fertilizer, the soaking of mustard green seed with Bacillus sp. is done for 20 minutes and the dose of organic fertilizer used 0.5 kg of plant -1 will provide the highest yield potential that is 24,490 ton ha-1 or lower 1,360 tonnes compared to 20 minutes immersion and the use of 1.5 kg ha-1 organic fertilizer is 25,850 ton ha-1. When converted to the needs of cost, then the addition of 1 kg of organic fertilizer plant-1, or about 40 tons ha-1 only able to add production of about 1,360 tons of mustard green. By increasing the cost of purchasing organic fertilizer 40 million rupiah can only get the results of 40 million and 480 thousand rupiah excluding freight costs and application fees. This suggests that mustard green plants require 0.5 kg of organic fertilizer plant-1, and if given more than that dose does not result in a significant increase in fresh weight.

Organic materials in addition to the effect on soil nutrient supply also to the physical, biological and chemical properties of the soil. Good soil physical condition can guarantee the growth of plant roots and able as a place of aeration and soil moisture. Organic matter plays an important role in the formation of good soil aggregates and structures, thus improving soil physics conditions, and ultimately facilitating water penetration, water absorption, root development, and increased resistance to erosion (Thompson and Goyne, 2012).

Soil organic matter determines the level of soil fertility, both chemical fertility, physics, and soil biology. This can be due to organic matter capable of contributing nutrients after decomposition, improving soil drainage, increasing infiltration, retention and transmission of water in the soil, and loosening soil and stabilizing soil aggregates, so that plant roots can thrive for nutrients and water for growth. The process of decomposition or mineralization, in addition to being influenced by the quality of organic matter, is also influenced by the frequency of adding organic matter, the particle size of the material, the drought, and the way it is used (mixed or dispersed on the surface) (Vanlauwe et al., 1997).

Conclusion

Treatment of green mustard seed soaking time and dose of organic fertilizer give real effect to the very real interaction on plant height, leaf number, fresh weight, oven dry weight, and plant fresh weight per hectare; but not significantly different for the root

length. The soaking of green mustard seed with Bacillus sp.as PGPR rhizobacteria for 10, 20 and 30 minutes combined with 1 kg or 1.5 kg organic fertilizer dose per plant gives no significant effect to plant growth parameters. The soaking of mustard green seed with Bacillus sp is done for 20 minutes and the dose of organic fertilizer used 0.5 kg of plant $^{-1}$ will provide the highest yield potential that is 24,490 ton ha^{-1} a combination treatment of the most effective, efficient and profitable.

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References

Antara M and Susrusa KB, 1991. Intensification of Quality of Vegetable Demand by Hotels and Restaurants of Badung Regency of Bali Province. Denpasar: Faculty of Agriculture Universitas Udayana Denpasar (in bahasa)

Antoun H and Kloepper JW, 2001. Plant growth–promoting rhizobacteria (PGPR). In: Brenner S, Miller JH (eds.) Encyclopedia of genetics. Academic, New York, pp 1477–1480

Araújo FF, Henning AA and Hungria M, 2005. Phytohormones and Antibiotics Produced by Bacillus subtilis and their effects on Seed Pathogenic Fungi and on Soybean Root Development. World J. Microbiol. Biotech. 21(8): 1639-1645.

Dwipayana HD, 2010. Identification of Bacterial Diversity in Waste Recycling Paint Sludge by Conventional Microbiological Technique. Environmental Engineering Study Program. Bandung.

McMilan S, 2007. Promoting Growth with PGPR. The Canadian Organic Grower. http//:www.cog.ca/

Poespodarsono S, 1998. Mustard plant. Malang: Faculty of Agriculture Universitas Brawijaya. (in bahasa)

Sarma MV, Saharan RK, Prakash K, Bisaria A and Sahai V, 2009. Application of Fluorescent Pseudomonads Inoculant Formulations on Vigna mungo through Field Trial. Int. J. Biol. Life Sci. 1(1): 25-29.

Sunaryono, 1994. Planting Vegetables Grow key important in Indonesia. Jakarta: Horticultural Research Institute Pasar Minggu Jakarta.(in bahasa)

Thompson A and Goyne KW, 2012. Introduction to the sorption of chemical constituents in soils. Nature Edu. Knowledge. 3(6): 15.

Van Peer R and Schippers B, 1988. Plant Growth Responses to Bacterization with Selected Pseudomonas Spp. Strains and Rhizosphere Microbial Development in Hydroponic Cultures. Can. J. Microbiol. 35: 456–463

Vanlauwe B, Diel J, Sanginga N and Merckx R, 1997. Residue quality and decomposition: An unsteady relationship. In Dirven by Nature Plant Litter Quality and Decomposition, (Eds. Cadisch, G. and K.E. Giller), pp. 157-166. Department of Biological Sciences, Wey College, University of London, UK.

Weller DM and Cook RJ, 1986. Increased Growth of Wheat by Seed Treatments with Fluorescens Pseudomonads, and Implications of Phytium Control. Can. J. Plant Pathol. 8:328-334.

Widnyana IK, Suprapta DN, Sudana IM and Rai Maya Temaja IG, 2013. *Pseudomonas alcaligenes* Potential Antagonist against *Fusarium oxysporum* f. sp. lycopersicum the Cause of Fusarium Wilt Disease on Tomato. J. Biol. Agric. Healthcare. 3(7): 163-169.

Widnyana IK and Javandira C, 2015. Activity Pseudomonas spp. and Bacillus sp. to stimulate Germination and Seedling Growth of tomato plants International Conference on Food, Agriculture and Natural resources (IC-FANRes, 2015) The University of Jember. August 31 - 2 September 2015. Agric. Agric. Sci. Procedia. 9: 419-4223.

Widnyana IK, Ngga M and Sapanca PLY, 2017. The Effect of Seed Soaking with Rhizobacteria *Pseudomonas alcaligenes* on the Growth of Swamp Cabbage (*Ipomoea reptans* Poir). OP Conf. Series: J. Physics: Conf. Series 95(2017): 12007.