The Role of Rizobacteria Pseudomonas Alcaligenes, Bacillus Sp. and Mycorrhizal Fungi in Growth and Yield of Tomato Plants

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International Journal of GEOMATE, Sept., 2019 Vol.17, Issue 61, pp. 174 -180
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2019.61.49587
Special Issue on Science, Engineering & Environment

THE ROLE OF RIZOBACTERIA *PSEUDOMONAS ALCALIGENES, BACILLUS SP.* AND *MYCORRHIZAL* FUNGI IN GROWTH AND YIELD OF TOMATO PLANTS

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ABSTRACT: The research aims to reduce the use of synthetic chemical fertilizers by utilizing microorganisms that can spur plant growth and also aims to improve the ability of tomato plant growth on dry soil through the utilization of mycorrhizal. This study begins by soaking the seeds of tomatoes for 20 minutes in a suspension of *P.alcaligenes* and *Bacillus* sp. with population density are 2.10^8 cfu and 3.10^8 cfu. The next tomato seeds seedling and planted in polybags already filled planting medium with *Mycorrhizal* or without *Mycorrhizal.* The results showed that soaking tomato seeds with suspense *P. alcaligenes* and *Bacillus* sp. or a combination of both types of bacteria are significant effect (P <0.05) on all parameters were observed, namely: tomato plant height, leaf number tomatoes, weight of fresh stems, weight and number of fruits, and root length of tomato plant. Similarly, the addition of *Mycorrhizal* significant effect (P<0.05) on all parameters observed. Tomato seed soaking treatment with suspense *P. alcaligenes* and *Bacillus sp.* can increase the number of leaves until 10.54%, 55.16% plant height, stem fresh weight 4.04%, fruit weight 344.44%, 162.83% fruit number, and root length of 17.90.% compared to control. The planting of tomato seed after soaking in suspense rizobacteria *P. alcaligenes* and *Bacillus sp.* on planting medium containing Mycorrhiza can increase the number of leaves up to 21.60%, plant height 7.67%, stem weight 30.62%, fruit weight 40.83%, fruit amount 30.04%, and length the roots of 35.03% compared with no addition of Mycorrhiza.

Keywords: Rizobacteria, Pseudomonas alcaligenes, Bacillus sp., Mycorrhizal., Tomato plants

1. INTRODUCTION

Tomato plants are one of the horticultural commodities which have high economic value and health-giving properties. The high content of vitamin C is good for improving the body's resistance to disease. To support the production of tomato plants, so that its availability is assured and can be produced through a healthy process, is needed studies on the cultivation of tomato plants thoroughly and deeply. Efforts to reduce the use of synthetic fertilizers are necessary in order to achieve sustainable agriculture that is environmentally friendly. More recently attention has begun to focus on biological resources in improving plant health, through the useful role of soil microbes. Microbes that are beneficial to plants, such as rizobacteria from the *Pseudomonas* sp. can function to fertilize the soil, as a means of biological control of plant pathogens and can improve plant resistance (*induced systemic resistance* ISR) [1]. Rizobacteria plays a direct role as biological fertilizers and biological stimulants by producing plant-growing hormones such as IAA (indole acetic acid), gibberellin, cytokinin, ethylene, dissolving minerals, and indirectly also preventing pathogenic microorganisms through the formation of siderophore and antibiotics [1]-[2]. *Pseudomonas spp.* provides a positive effect by occupying the surface of the plant's root tissue and providing the compounds favorable to the plant. Some of these bacteria enter further into the tissues and become endophytic without causing damage or morphological changes in plants [3].

The plant roots parts that have an influence on plant growth and development. The area around the plant is known to have a lot of nutrients including amino acids and sugars, nitrogen and carbon sources [4]. This plant root zone is called the rhizosphere. There are various microorganisms that are many in the region which began to fungi, bacteria, actinomycetes, and so forth. Bacteria have the ability of colonization root zone that is called Rhizobacteria. There are three bacteria's abilities to survive on plant roots, namely the ability of propagation, survive and grow into bacteria indigenous. There are several genera of bacteria that have interaction with plant roots, among others, *Acinetobacter, Agrobacterium, Arthrobacter, Azospirillum, Bacillus, Bradyrhizobium, Frankish, Pseudomonas, Rhizobium, Serratia, Thiobacillus* and so forth [5].

Rhizobacteria *Bacillus* sp. and *Pseudomonas* sp. known to have the ability to support plant growth. Capability Supports growth called with PGPR (*Plant Growth Promoting Rhizobacteria*) is when bacteria PGPR increase plant growth and plant resistance through the ability to produce PGR (plant growth regulators), phosphate solvent which can improve the efficiency of fertilizer phosphate production capability antibiotics, produce siderophores, which play a role in the induction of resistance or increase plant resistance to pest [6]. *Bacillus* sp. and *Pseudomonas* sp. an antagonist known microorganism used as a biocontrol agent against a disease that is contagious soil and air. These bacteria can produce compounds that are antibiosis as chitinase enzyme that can hydrolyze the cell wall of the fungus [7], siderophores, and other antibiotics that can inhibit the development of pathogens [8]. Mycorrhizal Vascular Arbuscular (MVA) is one type of soil fungus, whose presence in the soil has great benefits. This is because MVA can improve the availability and retrieval of phosphorus, water, and other nutrients, as well as for disease control caused by soil pathogens. MVA belongs to the group of Glomales that is an obligated parasite, so it cannot be inoculated with microbiological techniques but can be grown on live plant roots [9]. Mycorrhizal is a mutualistic symbiotic relationship between the fungus and the roots of the plant. Fungi and plants alike - equally benefit from this association. Fungal infections are useful, among others: (1) Helps plants absorb/take nutrients that are located further away from the roots of plants through hypa, (2) Better adaptation of plants in less favorable environments, (3) Mycorrhizal can meet the needs of life from plants Host. Based on that explanation, this study aimed at determining the role of the role rhizobacteria *Pseudomonas* sp. and *Bacillus* sp. pada tanah yang diinokulasikan dengan Mycorrhizale dan tanpa Mycorrhizal on the growth of tomato plants

2. MATERIALS AND METHODS

This research was conducted at the Laboratory of Agro Technology Faculty of Agriculture, University Mahasaraswati Denpasar and Experimental Farm Faculty of Agriculture, University Mahasaraswati from August 2015 - December 2015. The tools used in the study are Petri dishes, test tubes, Erlenmeyer, autoclave, laminar air flow, centrifuge, haemocytometer, hand counter, and EnCase. The materials used are 70% alcohol, spirits, aluminum foil, selective media Kings, B, PDA (Potato dextrose agar), NA (Nutrient Agar), isolates of *P. alcaligenes*, *Bacillus sp*. and *Mychorizzae sp*, tomato seeds, and distilled water.

2.1.Isolation Rhizobacteria P. Alcaligenes, Bacillus sp. and Mychorizzae fungi

Isolates of *P. alcaligenes* and *Bacillus* sp. obtained from previous research funded by ministries of research, technology and higher education in 2012-2013. Re-isolation of the bacteria *Bacillus sp* done using NA medium (Nutrient agar). To maintain the viability of the bacteria is then re-isolation made not later than 3 months [10].

Treatment of tomato seed immersion in a suspension of *P. alcaligenes* and *Bacillus sp.* do for 20 minutes. The bacterial population density was $2x10^8$ cfu, and $3x10^8$ cfu, with three types of bacterial suspense *P. alcaligenes*, *Bacillus sp.*, and mixtures of both types of bacteria. Planting of tomato seeds is divided into 2 types that are grown on growing media with Mycorrhizal and without Mycorrhizal. Mycorrhizal fungi used in this study is the production of Bogor Agricultural Institute. The treatment was repeated 3 times so that there were 36 experimental units (3x2x2x3). Parameters measured were: height and number of tomato leaves, fresh weight of tomato stems, amount and weight of fresh tomato fruit.

3. RESULTS AND DISCUSSION

3.1. The Effect of Treatment on the Number of Tomato Leaves

The average number of tomato leaf at week 8 (eight) on treatment of soaking with *P. alcaligenes* and *Bacillus sp*. and planted on planting media containing mycorrhizal fungi are presented in Table 1, which shows that the most number of leaves occur in the treatment of soaking of tomato seeds in a mixture of bacterial suspension of *P. alcaligenes* with *Bacillus sp.* but not significantly different in *P. alcaligenes* alone, which is an average of 32.7 leaf. This means the soaking of tomato seeds with suspense *P. alcaligenes* gives the same effect singly or when mixed with *Bacillus sp.* The addition of mycorrhiza can increase the number of leaves of almost all tomato seed immersion treatments, but not significantly influence the treatment of tomato seed immersion in the population density of *Bacillus sp.* $2x10^8$ cfu.

Table 1. The Number of Tomato Leaves in the PGPR Population Density Treatment on the Media Contained Mycorrhizal and Without Mycorrhizal

Treatment	The number of tomato leaves at week 8		increase the number of leaves (%)
	without mycorrhizal	with mycorrhizal	
Control	28.3 c	41.0 a	44.87
P. alcaligenes 2 x10^8cfu	32.7 a	34.0 c	3.97
Bacillus sp. 2 x10^8cfu	29.3 c	32.0 de	9.21
P. alcaligenes + *Bacillus sp.*2 x10^8cfu	32.7 a	33.0 cd	0,90
P. alcaligenes 3 x10^8cfu	31.0 b	37.7 b	21.60
Bacillus sp.3 x10^8cfu	29.3 c	31.0 e	5.80
P. alcaligenes + *Bacillus sp*. 3 x10^8cfu	32.7 a	34.3 c	4.90
LSD 5%	1.13	1.49	

3.2. The Effect of Treatment on High and Fresh Weight of Tomato Plants

Effect of Mycorrhiza, *P. alcaligenes* and *Bacillus sp.* to the height of tomato plants at week 8 (eight) is presented in Table 2. Table 2 shows that the highest tomato plants without Mycorrhizal treatment were found in the tomato soaking treatment with *Baccillus sp*. 3x10^8 significantly different ($P< 0.05$) with all other treatments, and the lowest was in the control plants.

Table 2. The Height of Tomato Plants in the PGPR Population Density Treatment on the Media Contained Mycorrhizal and Without Mycorrhizal

Treatment	The average plant height at week 8 (cm)		increase the plant height (%)
	without mycorrhizal	with mycorrhizal	
Control	84.0 c	132.0 c	57.14
P. alcaligenes 2 x10^8cfu	120.7 b	122.0 d	1.07
Bacillus sp. 2 x10^8cfu	130.3 b	140.3 b	7.67
P. alcaligenes + *Bacillus sp.* 2 x10^8cfu	129.3 b	134.0 c	3.63
P. alcaligenes 3 x10^8cfu	130.3 b	140.0 b	7.44
Bacillus sp.3 x10^8cfu	141.7 a	144.0 a	1.62
P. alcaligenes + *Bacillus sp*. 3 x10^8cfu	129.7 b	139.0 b	7.17
LSD 5%	2.63	2.43	

In all observations, the height of tomato plants in seeds soaked with rhizobacterial suspension and grown on the inoculated medium of mycorrhizae is 1.07 - 7.67% higher than that grown on a medium not inoculated Mykoriza. The effect of single mycorrhizal inoculation caused tomato height increased 57.14% compared without mycorrhizal inoculation. This suggests that the addition of Mycorrhizal plays a significant role in the addition of tomato plants.

Influence of mycorrhizal inoculation, *P. alcaligenes* and *Bacillus sp.* to the fresh weight of tomato plants are presented in Table 3. The planting of tomato seeds that have been soaked in rhizobacteria suspension on Mykoriza inoculated planting media gives a significant effect on the fresh weight of tomato plants. Table 3 shows that the highest fresh weight of tomato plants was found in the treatment of suspended mixtures of *P. alcaligenes* with *Bacillus sp*. 2x10^8 cfu of 333.3 g and significantly different ($P <0.05$) with all other treatments. However, after the addition of Mycorrhizal, the highest increase in fresh weight of tomato plants occurred in the treatment of soaking of tomato seeds with Bacillus sp. 3x10^8 cfu increased by 30.62%, followed by treatment with a mixture of suspensions of *P. alcaligenes* and *Bacillus sp.* 3x10^8 cfu that is increased 24.12%.

Table 3. The Fresh Weight of Tomatoes Fruits in the PGPR Population Density Treatment on the Media Contained Mycorrhizal and Without Mycorrhizal

Treatment	Fresh weight of tomatoes fruits (grams)		Increase fruit weight (%)
	without mycorrhizale	with mycorrhizale	
Control	100.0 c	300.0 c	200.00
P. alcaligenes 2 x10^8 cfu	483.3 a	506.7 b	4.84
Bacillus sp. 2 x10^8 cfu	408.3 b	575.0 a	40.83
P. alcaligenes + *Bacillus sp.* 2 x10^8 cfu	433.3 b	608.3 a	40.39
P. alcaligenes 3 x10^8 cfu	425.0 b	491.7 b	15.69
*Bacillus sp.*3 x10^8 cfu	425.0 b	483.3 b	13.72
P. alcaligenes + *Bacillus sp.* 3 x10^8 cfu	491.7 a	516.7 b	5.08
LSD 5%	31.74	42.19	

3.3. The Effect of Treatment on Tomato Length Root

In Table 4. are presented the effect of population density *P. alcaligenes* and *Bacillus sp.* to tomato plant roots. The shortest tomato roots on treatment without mycorrhizal in control plants at 27 cm, the longest roots are in the treatment of *P.alcaligenes* 2x10^8 cfu is 40.00 cm or 32.5% longer than the control. The most interesting thing is the control (without rizobacteria) with growing media containing mycorrhizal increased root length up to 40.00% from 27.00 cm to 45.00 cm. This indicates that Mycorrhizal plays an important role in the addition of water absorption and nutrients to plant roots.

Table 4. The Mean Number of Tomatoes Fruits in the PGPR Population Density Treatment on the Media Contained Mycorrhizal and Without Mycorrhizal

Treatment	The average number of fruit tomato		increase the number of fruits (%)
	without mycorrhizale	with mycorrhizale	
Control	10.0 b	25.0 b	150.00
P. alcaligenes 2 x10^8 cfu	25.7 a	27.3 ab	6.22
Bacillus sp. 2 x10^8 cfu	32.7 a	35.0 a	7.03
P. alcaligenes + *Bacillus sp.* 2 x10^8 cfu	23.3 a	30.3 ab	30.04
P. alcaligenes 3 x10^8 cfu	24.3 a	28.0 ab	15.23
*Bacillus sp.*3 x10^8 cfu	24.7 a	28.0 ab	13.36
P. alcaligenes + *Bacillus sp.* 3 x10^8 cfu	27.0 a	32.3 ab	19.63
LSD 5%	10.23	9.46	

3.4. The Effect of Treatment on Tomato Fruits

The treatment of tomato seeds soaking with the suspension of *P. alcaligenes* and *Bacillus* sp. significant ($P<0.05$) to the weight of fresh tomatoes, as well as the addition Mycorrizhal significant effect ($P<0.05$) to the weight of fresh tomatoes. More clearly presented in Table 5. Table 5 shows the highest production of fresh tomatoes found in the treatment of suspense mixture of *P. alcaligenes* with *Bacillus sp.* 3x10^8 cfu of 491.7 g/plant was not significantly different from *P. alcaligenes* 2x10^8 cfu treatment ie 483.3 g/plant. The highest fresh tomato fruit production after added Mycorrhizale was found in the mixed treatment of *P. alcaligenes* suspension with *Bacillus sp.* 2x10^8 cfu of 608.3 g/plant and not significantly different from *Bacillus sp.* 2.10^8 cfu ie 575.0 g/plant.

Table 5. Effect of PGPR Population Density Treatment on Root Length of Tomato on Planting Medium Contained Mycorrhizal and Without Mycorrhizal

Treatment	The average root length of tomato		increase the number of fruits (%)
	with out mycorrhizale	with mycorrhizale	
Control	27.00	45.00	40.000
P. alcaligenes 2 x10^8 cfu	40.00	40.67	1.639
Bacillus sp. 2 x10^8 cfu	29.67	39.00	23.932
P. alcaligenes + *Bacillus sp.* 2 x10^8 cfu	34.33	35.33	2.830
P. alcaligenes 3 x10^8 cfu	30.00	37.33	19.642
*Bacillus sp.*3 x10^8 cfu	34.00	52.33	35.032
P. alcaligenes + *Bacillus sp.* 3 x10^8 cfu	23.00	29.33	21.591
Total	600	747	

The treatment of soaking of tomato seeds with rizobacteria *P. alcaligenes* and *Bacillus sp* gave a significant effect ($P < 0.05$) on the number of tomatoes both on treatment with mycorrhizale or without the addition of mycorrhizal. In the treatment without Mycorrhizal there was no significant difference ($P > 0.05$) on the number of tomatoes between soaking treatments with *P.alcaligenes* and *Bacillus sp.* However, a significant improvement occurs in only with Mycorrhizal treatment of 150%. More clearly presented in Table 5.

3.5. Discussion

Colonization of rhizosphere by *Pseudomonas spp.* strains causing the acceleration of plant growth or protection against plant pathogens, leading to maximum plant growth [11]. Large variations were observed in the percentage of rhizobacteria antagonistic to selected soilborne root pathogens in vitro. Although antagonistic rhizobacteria were found in the control treatment. The rhizobacteria which were from compost 2 contained the highest percentage of rhizobacteria antagonistic to these phytopathogens. Although the antibiosis test in vitro does not always correlate with suppression of soilborne plant disease, because of the magnitude of the rhizosphere population and the lack of a more reliable method, in vitro screening of potential antagonists is valuable [12].

Pseudomonas spp. group is known to be beneficial to plants. Some strains have long been known as a biological control agent [13]-[14], vanilla stem rot disease control *Fusarium oxysporum* f.sp. *vanillae* [15], control of tomato wilt disease *Fusarium oxysporum* f.sp. *lycopersicum* [16]. The bacteria are also known as plant growth promoting rhizobacteria (PGPR), either directly or as a result of its ability to control the disease [17]-[18].

According to Tenuta [19], PGPR bacterial mechanism in improving the health of plants can occur in three ways, namely: a). Decrease development of pest / disease (bioprotectant): has a direct influence on the plant in the face of pests and diseases, b) producing phytohormones (biostimulant): IAA (Indole Acetic Acid); cytokinins; giberellin; and inhibiting the production of ethylene: can increase the surface area of fine roots, c) increasing the availability of nutrients for plants (biofertilizer). Meanwhile, according to Mc. Milan [1], several roles PGPR in spurring the growth of the plants: (a) increase nitrogen fixation in legumes, (b) increase the population of bacterial fixing nitrogen (c) boost the supply of other nutrients, such as phosphorus, sulfur, iron and copper, (d) hormone production, (e) increase the population of beneficial fungi or bacteria, (f) control of fungal pathogens, (g) controlling pathogens as bacteria and (h) to control insect pests.

Plant resistance can be divided into resistance to which it has been formed prior to pathogen attack plants (pre-exiting) and plant resistance-induced by an agent (induced resistance). Resilience pre-exiting can be broken if it is infected by a pathogen that is virulent because the pathogen is able to overcome plant resistance reactions. However, when the defense mechanisms are triggered by agents of stimulants (PGPR) prior to infection by pathogens, the severity of the disease will decrease [20]. Rizosphere colonization by strains of *Pseudomonas* spp causing the stimulation of plant growth or protection against pathogens plants, resulting in maximum plant growth [10].

According to Gunawan [21], term Mycorrhizal vesicular-arbuscular (VAM) is used for all fungi of the types of fungus order Glomales can form the structure of arbuscular in association with roots and is only partially able to form vesicular. Vascular arbuscular mycorrhizal (VAM) is one type of soil fungus, which exists in the soil so it has a benefit. This is because the VAM can improve the availability and retrieval of phosphorus, water, and other nutrients and for the control of diseases caused by pathogenic soil borne. VAM Glomales classified into orders that are obligate parasites, so it cannot be inoculated with microbiology techniques. However, it can be grown on living plant roots [9]. Further stated that, the role of which is more detailed than mycorrhizale on plant growth are 1) Mycorrhizale can effectively improve the absorption of nutrients both macronutrients, especially P (Phosphorus) and micronutrients, 2) mycorrhizal can absorb nutrients in the form of bound and not available to plants, 3) mycorrhizal can interact positively with various types of soil microorganisms both symbiotic and free-living as fixing bacteria N symbiotic and non-symbiotic bacteria phosphate solvent 4) plants whose roots with mycorrhizale more resistant to drought than those without mycorrhizale, 5) In the crop with mycorrhizale roots by mycorrhizall root cause protected from pests and disease or pathogen infection root hampered besides that there mycorrhizall fungi that can release antibiotics that can kill pathogens, and 6) mycorrhizale can produce growth hormone such as cytokinin and gibberellin and plant growth regulators such as vitamin can also be produced by some fungi mycorrhizale.

The use of mycorrhizal is more interesting in terms of ecology because it is safe to use and does not cause environmental pollution. If a particular mycorrhizall species develop in cropland then its benefits will be forever. In this study of mycorrhizall fungi, mycorrhizale applied by placing it around the roots of tomato plants. The interaction between the host plants with mycorrhizale has an influence on plant growth and on an ecosystem. Simarmata *et al.* [22] states that the effectiveness of mycorrhizall fungi are closely related to various environmental factors soil abiotic (nutrient concentrations, pH, water content, temperature, tillage, and the use of fertilizers/pesticides) and biotic factors (microbe interactions, species of fungi, host plants, and competition between mycorrhizal fungi).

4. CONCLUSIONS

The results of this study indicate that soaking of tomato seeds with the suspension of bacteria *P. alcaligenes* and *Bacillus sp*. particularly helpful in improving the growth and yield of tomato plants, either singly or a mixture of both rizobakteri the suspense. The population density of bacteria *P. alcaligenes* and *Bacillus sp* in 2.10^8 and 3.10^8 have the same effect on the growth and yield of tomato. However, tomato plant yield will increase if also added the addition of Mychorriza on tomato plant growth medium. If there is no rizobacterial P. alcaligenes and Bacillus sp., the addition of Mychorriza singly is sufficient to obtain the appropriate growth and yield of tomatoes.

5. Acknowledgment

Acknowledgments are presented to the Ministry of Research and Technology of Higher Education of the Republic of Indonesia, Directorate of Research and Community Empowerment which has funded research through the National Competitive Research Grant.

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