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# Growth and yield of green bean (*Vigna radiata* L.) as affected by *Rhizobium* sp. bacteria inoculant and frequence of watering

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Abstract. The aim of the study was to know the influence of the *Rhizobium* bacteria inoculant doses as biotic fertilizer and the frequence of watering on the growth and yield of the green bean (*Vigna radiata* L.). The study conducted in Green House of Faculty Animal and Agricultural Sciences, Diponegoro University. The experimental design of this research was Completely Randomized Design (CRD) factorial with two factors namely dose of *Rhizobium* bacteria inoculant (R) which consist of R0 = control, R1 = possitive control (1,8 mg/ plant of commercial inoculant), R2 = 5 mg/ plant inoculant of *Rhizobium*, R3 = 10 mg/ plant inoculant of *Rhizobium* and R4 = 15 mg/ plant inoculant of *Rhizobium* while frequence of watering (F) which consist of five standards that F1 = a time in a day, F2 = a time in two days and F3 = a time in three days. With 3 replications. The parameters observed were height of plant, number of leaves, days of blooming, and days of pod. The result showed that the dose of the *Rhizobium* inoculant was significantly affected by the days of blooming. The frequency of watering significatly affected on height of plant, number of leaves, days of the plant. There were significant interaction effect on the days of the bloom and days of the podded. Key words: green bean, *Rhizobium*, watering frequence, growth, yield.

#### 1. Introduction

Green bean is a plant which is from the legume class. Green bean is an important commodity of legume plants after soybeans and peanuts. Compared with other types of legumes, green beans have the advantage of agronomic and economic aspects, namely, fewer pests and diseases, can be harvested at the age of 55-60 DAP and a higher selling price than soybeans (Barus *et al.*, 2014) Green bean are the most widely used by humans. Improving the processed industry made from green beans makes the demand for green beans in Indonesia continue to increase. The high demand has not been accompanied by the availability of green beans supply by farmers. In 2015 until 2017, the production and harvest area of green beans were decreased, respectively 3.57% and 7.36%. Increasing green bean production can be optimized by using less productive lands, one of which is dry land. Water-deficient soil conditions can cause disruption to plant physiology and microorganisms in the soil. One technology that is expected in dealing with drought conditions is the use of *Rhizobium* sp. bacteria as biological fertilizer.

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Global climate change which has an impact on drought also becomes one of the obstacles in the cultivation of green beans. Drought is a condition where water needs are not met, so that it will affect plant metabolism, including microorganisms associated with these plants. In general, inoculation of *Rhizobium sp.* into the ground, intended to be able to associate with plants, thereby increasing the ability to bind N in the air. Therefore, it is necessary to test the association of *Rhizobium sp.* in green bean plants in soil conditions with different levels of drought.

### 2. Materials and Methods

The research conducted in Green House of the Faculty of Animal and Agricultural Science, Diponegoro University, Semarang and continued with laboratory analysis at the Laboratory of Ecology and Plant Production, Faculty of Animal and Agricultural Science, Diponegoro University. The experimental design of this research was Completely Randomized Design (CRD) factorial with two factors namely dose of *Rhizobium* sp. bacteria inoculant (R) which consist of R0 = control, R1 = contpossitive control RHIZOKA (1,8 mg/ plant of commercial inoculant), R2 = 5 mg/ plant inoculant of Rhizobium, R3 = 10 mg/ plant inoculant of Rhizobium and R4 = 15 mg/ plant inoculant of Rhizobium while frequence of watering (F) which consist of five standards that F1 = a time in a day, F2 = a time in two days and F3 = a time in three days. With 3 replications. The material needed is green bean variety VIMA-3, isolate of Rhizobium sp. (from green bean roots in Demak), ingredients for making 1 liter of Yeast Mannitol Agar (YEMA) + Congo Red (CR) medium consisting of 0.5 g K<sub>2</sub>HPO<sub>4</sub>; 0.2 g MgSO4; 0.1 g NaCl; 10 g Mannitol; 1 g of yeast extract; 20 g of bacto agar; 2.5 ml of Congo Red 1%. Materials for making liquid media of Rhizobium sp., peat, NPK fertilizer, soil. The tools needed are 30 cm diameter polybags for growing green bean plants, barrels for planting media sterilization containers, stoves for heating equipment, hoes to mix and move planting media to polybags, sieves to filter carrier media, stirers to mix ingredients for making media to grow bacteria, autoclaves for sterilizers, scales to measure the volume of material to be used, lamina air flow for the multiplication of Rhizobium sp., test tubes for bacterial dilution containers and the isolation of Rhizobium sp., erlenmeyer for liquid media containers, bunsen for sterilizing tools, analytical scales for weighing, rulers for measuring plant height, and ovens for removing moisture and sterilization, ose for taking bacterial isolates, shakers for mixing bacterial liquid media.

### 3. Results and Discussion

The presence of *Rhizobium* sp. bacteria associated with root nodules is influenced by environmental factors. *Rhizobium* sp. Bacteria. live at optimum environmental conditions. According to Surtiningsih (2009) which states that the Rhizobium sp. live at a temperature of  $18-26^{\circ}$  celsius, a minimum temperature of  $3^{\circ}$  celsius and a maximum temperature of  $30^{\circ}$  celsius. Temperature in the soil is influenced by the temperature and water received by the soil. This experiment examines the effect of Rhizobium sp. with different watering frequency.

Based on the analysis it is known that the treatment of watering frequency has a significant effect while the rhizobium inoculum dose treatment and the interaction of both have no significant effect.

 Table 1. Height of Plant of Green Bean that Innoculated with Different Dosage of *Rhizobium* sp. Bacteria and Different Frequence of Watering

Rhizobium Inoculant	Height of Plant					
Bacteria	F1	F2	F3	Mean		
R0 (control)	50.66 ab	44.00 abcde	36.66 cdef	43.78		
R1 (possitive control)	43.33 abcde	40.33 bcdef	32.66 ef	38.78		
R2 (5 mg/ plant)	54.00 a	42.66 abcdef	33.00 ef	43.22		
R3 (10 mg/ plant)	49.33 ab	42.00 bcdef	34.00 def	41.78		

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R4 (15 mg/ plant)	46.66 abc	44.66 abcd	31.33 f	40.89
Mean	48.80 a	42.73 b	33.53 c	(-)



Figure 1. Green bean in interaction treatment

Table 1. and Figure 1. showed that the tallest of the height plant is F1 treatment (48.80 cm), and F3 (31.53 cm) is the shortest. Perlakuan F1 berbeda nyata dengan perlakuan F2 dan F3. The height of green beans planted every day is higher than that of plants that are watered every other day and every three days. Daily watering treatment makes the plant height higher by 12.4% and 31% in F2 and F3, respectively. Green bean plants that are watered once a day have the highest value. Research by Daba and Tadese (2018) [2] concluded that Moringa olifera plants that are watered with sufficient water capacity consistently every day have optimum growth compared to those that are not.

From Table 1. it is known that the plant height in the Rhizobium dose treatment was not significantly different. Green bean plant height given inoculum Rhizobium sp. with various doses or without given an inoculum there is no difference. From Table 1. we know that there is no significant interaction between the treatments that are given.

Table 2. Number of Leaves of Green Bean that Innoculated with Different Dosage of Rhizobium sp. Bacteria and Different Frequence of Watering

Rhizobium Inoculant		Numbe	r of Leaves		Commented [DH14]: Experimental groups
Bacteria	F1	F2	F3	Mean	
R0 (control)	5.00 a	5.00 a	4.33 ab	4.78 a	
R1 (possitive control)	5.00 a	4.66 ab	3.66 abc	4.44 ab	
R2 (5 mg/ plant)	4.66 ab	4.00 abc	3.00 c	3.89 b	
R3 (10 mg/ plant)	5.00 a	4.33 abc	4.33 ab	4.44 ab	
R4 (15 mg/ plant)	5.00 a	4.66 ab	3.33 bc	4.33 ab	
Mean	4.93 a	4.47 a	3.73 b	(-)	Commented [DH15]: Write the SUPERSCRIPT prope

From Table 2. it is known that the frequency of watering in F1 and F2 treatments did not differ, while the F1 treatment was significantly different from F3 and F2 treatment was significantly different from F3. The number of leaves on green bean plants that are watered every day has a number of leaves more than 24% compared to green beans plants that are watered once every 3 days. According to erly

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Kalaydjieva et al. (2015) [3] showed that irrigation influences the leaf growth of Phaseolus vulgaris plants. Water as a photosynthetic material, if the amount is reduced, it will inhibit photosynthetic synthesis. This can inhibit the process of growth and formation of organs from plants.

Based on Table 2. it is known that the administration of different dosage of inoculum does not have a significant effect on the number of leaves of green bean plants. The results of the statistical analysis showed no interaction of the dose of Rhizobium sp. Inoculum. and frequency of watering on the number of leaves of green bean plants.

Table 3. Day of Blooming of Green Bean that Innoculated with Different Dosage of Rhizobium sp. Bacteria and Different Frequence of Watering

Rhizobium Inoculant		Days o	f Blooming		Commented [DH16]: Experimental groups
Bacteria	F1	F2	F3	Mean	
R0 (control)	39.66 ab	41.66 ab	40.33 ab	40.56 a	-
R1 (possitive control)	40.00 ab	40.00 b	42.33 ab	40.78 b	
R2 (5 mg/ plant)	36.66 b	36.66 b	43.00 a	38.78 ab	
R3 (10 mg/ plant)	38.00 b	43.33 ab	35.33 b	38.89 a	
R4 (15 mg/ plant)	40.00 b	39.33 b	45.33 a	41.56 b	
Mean	38.87 b	40.20 b	42.27 a	(-)	<b>Commented [DH17]:</b> same comment and suggestion

From Table 3. it is known that the time of emergence of plant flowers treated with watering frequency treatment in F1 (39 HST) and F2 (40 HST) treatments did not differ, whereas F1 treatment was significantly different from F3 (42 HST) and F2 treatment was significantly different from F3. When the flowers appear on the green bean plants that are watered every day and every two days have a shorter flowering time than the green beans that are watered every 3 days.

The results of the statistical analysis showed that there was an interaction of Rhizobium sp. and the frequency of watering when the green bean flower plants appear. The fastest time to appear was R3F3 (35 HST) treatment while the longest time to appear was R4F3 (45 HST). Green bean plants which are treated with Rhizobium 5 mg inoculum / plants watered every three days, have a flower time that is equivalent to the green bean plants that are applied with RHIZOKA with watering every day.

Based on Table 3. it is known that the administration of different dosage of inoculum does not have a significant effect on the flowering time of green bean plants. Administration of Rhizobium sp. increasing nitrite so that it can be used by legume plants, but nitrite is not dominantly used in generative growth such as flower formation. According to Farias et al. (2016) [5] which stated that the administration of Rhizobium inoculums did not give significant effect during the flowering period of the Vigna unguiculata plant.

Table 4. Days of Podded of Green Bean that Innoculated with Different Dosage of Rhizobium sp. Bacteria and Different Frequence of Watering

Rhizobium Inoculant	Days of Podded				Commented [DH18]: Treatment Group
Bacteria	F1	F2	F3	Mean	
R0 (control)	38.33 c	43.66 abc	40.00 bc	40.66 ab	
R1 (possitive control)	43.33 abc	41.66 abc	45.00 abc	43.33 b	
R2 (5 mg/ plant)	39.66 bc	39.00 c	44.00 abc	40.88 ab	

as table 2

R3 (10 mg/ plant)	40.33 bc	44.33 abc	49.66 a	44.77 a
R4 (15 mg/ plant)	40.33 bc	40.00 bc	48.33 ab	42.88 b
Mean	40.40 b	41.733 b	45.40 a	(-)

From **Table 4.** it is known that the time of emergence of plant pods treated with watering frequency treatment in F1 (40 HST) and F2 (42 HST) treatments did not differ, while F1 treatment was significantly different from F3 and F2 treatment was significantly different from F3 (45 HST). When the pods appear on the green beans plant watered every day and every two days have a shorter time of appearing pods than the green beans plant watered once every 3 days.

The results of the statistical analysis showed that there was an interaction of Rhizobium sp. and the frequency of watering when the pods appear in the green bean plants. The fastest time for pods to appear was R0F1 (38 HST) treatment while the longest pods appeared was R3F3 (50 HST) treatment. When pods appeared in R2F3 plants were not significantly different or the same as the treatment of R1F1, R2F1, R3F1 and R4F1. Green bean plants which were applied with Rhizobium sp. 5 mg / pot with watering every three days has a pod emergence time equivalent to the treatment without Rhizobium application, RHIZOKA inoculum application and Rhizobium inoculum application that is watered every day. According to Ntambo *et al.* (2017) [4] which states that Rhizobium bacteria inoculation can increase the appearance of pods in soybean plants, this is related to increased nitrite intake so that plants can survive in unfavorable conditions.

Based on **Table 4.** it is known that the different dosage of inoculum does not have a significant effect on the time the pods appear on the green bean plant.

**Table 5.** Weight of Pods (g) of Green Bean that Innoculated with Different Dosage of *Rhizobium* sp. Bacteria and Different Frequence of Watering

Rhizobium Inoculant	Weight of Pods (gram)					
Bacteria	F1	F2	F3	Mean		
R0 (control)	19.10 a	11.50 bcd	15.83 ef	15.80		
R1 (possitive control)	15.30 ab	9.17 bcd	2.50 f	9.00		
R2 (5 mg/ plant)	14.00 abc	5.50 de	2.00 f	7.17		
R3 (10 mg/ plant)	13.80 ab	7.67 dc	3.00 ef	8.17		
R4 (15 mg/ plant)	11.67 abcd	12.50 abcd	1.30 f	8.50		
Mean	14.80 a	9.27 b	4.93 c	(-)		

From **Table 5.** showed that frequence of watering treatment has interaction with weight of pods. It is known that weight of pods treated with watering frequency treatment in F1 (14.80 gram) was significantly different from F2 (9.27 gram) and F3 (4.93 gram) treatment.

The results of the statistical analysis showed that there was not an interaction of *Rhizobium* sp. and the frequency of watering in the weight of the green bean pods. Based on **Table 4.** it is known that the different dosage of inoculant also didn't have a significant difference for weight of pods in green bean. Based on research by Hussain *et al.* (2014) [6] said that phosphorus needed more that nitrogen when developing of pods.

## 4. Conclusion

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Result showed that the dose of the *Rhizobium* sp. inoculant significantly affected days of blooming and days of podded. The frequency of watering signifactly affected on height of plant, number of leaves, days of the bloom, days of the podded and weight of pods. There were significant interaction effect on the days of the bloom and days of the podded. Green bean that treatmented by *Rhizobium* sp. bacteria inoculant and a time in three days has same in days of blooming and days of podded with bacteria inoculant RHIZOKA. The adviced for using *Rhizobium* sp. inoculant to make faster blooming and poding is 5 mg/ plant.

#### Acknowledgments

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