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Research articles

Effect of inoculation and time of application of microbes on growth and yield of soybean (*Glycine max* (L.) Merrill)

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Abstracts: The application of inorganic fertilizers continuously can result in hardening of soil, narrowing of soil pores, thus the roots will have difficulty in penetrating soil. Biological properties of soil that decreases could lead to disruption of microorganism activity, so that the decomposition of organic matter of soil become obstructedand soil fertility decline. Therefore, it is advisable to use an alternative to application of microbes, i.e. *Rhizobium* and effective microorganisms 4 (EM4) that will enhance the availability of nutrients for plants. This study was aimed to determine the effect of inoculation and time of application of the microbes on the growth and yield of soybean. The experiment was conducted in the greenhouse located 500 m above sea level. The design used for the experiment was a completely randomized design with three replications. Observations of non-destructive and destructive data were analyzed by analysis of variance (F test) at 5% level and continued with LSD at 5% level. The results showed significant effect treatments applied on the growth and yield components. Treatment i6 (EM4 applied at 1 week after planting) was the best treatment, as shown by the increased number of pods by 95.94%, dry weight of pods by 38.25%, total dry matter, seed by 24.61%, and 68.40% weight of 100 seeds.

Keywords*: effective microorganisms, fertilizer, Rhizobium, soybean*

Introduction

Soybean (*Glycine max* (L.) Merrill) is one of three major food commodities after rice and maize to become a strategic commodity on economic development in Indonesia. Soybean is a versatile plant because its roots have nitrogen-fixing free nodules (Wieta, 2008).

The decline in soybean production can be detrimental to farmers due to the use of inorganic fertilizers that gradually makes hard soil and compacted soil pores, so that the roots will have difficulty to penetrate the soil and disturb nutrient balance (Pranata, 2010). In addition, the excessive use of inorganic fertilizer candecrease soil biological characteristics which resulting in impaired activity of microorganisms. Thus, the process of decomposition of soil organic material and its fertility will decline (Cahyono, 2003). On the other hand, the farmers seemed to be very dependent on the use of inorganic fertilizers, although the price of an-organic fertilizer is very high and sometimes are rare in some areas of agricultural centers (Suryo, 2006). Therefore, it is now recommended to seek an alternative to the inorganic fertilizer by giving , i.e. *Rhizobium* and effective microorganisms 4(EM4) for accelerating the availability of nutrients for plants, increasing the yield and quality of crops at low costs through labour and chemical fertilizers (Saraswati and Sumarno, 2008).

Rhizobium is a bacterium that colonizes onroot zone that can provide benefits toplants for stimulatingplants growth and controlling disease biologycally. According to Shutsrirung et al. (2002), more than 60% N of the required soybean plants can be supplied through symbiosis with *Rhizobium*. Likewise, according to Noortasiah (2005), the provision of *Rhizobium* can minimize N fertilizer to 22.5 kg N/ha. This shows that *Rhizobium* inoculantion is capable in doing symbiosis actively to enhance plant growth.

Rahayu (2004) reported that the giving of *Rhizoplus* on soybean of Willis variety could improve plant growth, as the number of branches per plant, number of pods per plant and yield per ha. Further, Novriani (2011) reported that the use of *Rhizobium* that is one of environmentally friendly farming technologies is sustainable and good for use in the production of soybean. The result results of Adijaya et al. (2004) showed that the use of Legin (*Rhizobium*) on soybean cultivation could improve the components of growth and expand the nodule on soybeans. Furthermore, with the increasing growth and the number of nodules, *Rhizobium* symbiosis in fixing free N from the air also increased. This would lead to increase availability of N for plants that affected the increase of soybeanproduction . Ningsih and Anas (2004) research showed that *Rhizobium* inoculation and IAA were able to improve plant growth, root nodule formation and nutrient uptake of soybean plants.

Utilization of EM can improve soil quality and further improve plant growth and crop production. EM can dissolve nutrients from parent material with low solubility, react a heavy metal into a compound for the inhibition of absorption of heavy metals in plant roots, provide simple organic molecules that can be absorbed directly by the plant, stimulate the growth of plants by releasing plant growth regulators, and improve structure/decomposition of organic matter and plant residues so that accelerate the process of recycling nutrients in the soil. Microorganisms contained in EM4 are beneficial microorganisms consisting of lactic acid bacteria, photosynthetic bacteria that can be synergisticwith *Rhizobium sp.* (Pasaribu et al., 1989), actinomycetes, yeasts and fungi. These beneficial microorganisms actively control the microorganisms present in the soil and improve soil fertility. Thus, it will be beneficial to the growth and crop production (Kristasi, 1999).

Based on above description, it is needed to do research on microbial inoculation and time of application of microbes microbes, i.e. *Rhizobium* and effective microorganisms 4 (EM4) on the growth and yield of soybean (*Glycine max* L. Merrill).

Materials and Methods

This research was conducted from June to November 2011 at the greenhouse of the Faculty of Agriculture, Universitas Islam Malang which is approximately located at 500 m above sea level. The materials used in this study were seeds of soybean of Wilis variety, *Rhizobium*, EM4, waste of fungus media, and sand.

The study was conducted using a completely randomized design with the following treatments: I_0: control (no treatment), i_1: *Rhizobium* 1 week aplication before planting, i_2: *Rhizobium* application at planting time, i_3: *Rhizobium* application 1 week after planting, i_4: EM4 1 week application before planting, i_5: EM4 application at planting time, i_6: EM4 application 1 week after planting, i_7: *Rhizobium* and EM4 application 1 week before planting, i_8: *Rhizobium* and EM4 application at planting time, i_9: *Rhizobium* and EM4 1 weeks application after planting. Each treatment was repeated three times. There were thirty experimental units, seven number of samples per unit of experiment with 210 total plants.

The planting medium used was waste from fungus media that has been previously dried. The waste was then mixed with sand with the ratio of 1:1. Before mixing, the two materials were sieved with a diameter of 2 mm then mixed evenly on both growing media materials. The mixture was then placed into a polybag with a weight of 10 kg/polybag. The planting media was supplied with water to field capacity. The application of microbial treatment was done 1 week before planting, at the same time to the preparation of planting medium.

Microbial inoculation (*Rhizobium* and EM4) was made by diluting the *Rhizobium* as recommendation (5 g/L of water) and EM4 (50 mL/L of water), and then mixing the microbial solution to the growing media that has been moistened beforehand. The application times were 1 week before planting, at planting time and 1 week after planting.

Observations of growth and harvest were done in two ways; observation of non-destructive in terms of height, number of leaves, leaf area, number of productive branches, flowering time, and the amount of flowers and destructive observations include the number of nodule, pods total/plant, number of pods with seeds/plant, dry weight of pods with seeds/plant, total dry weight of seed/plant, 100-seed weight and dry weight of stover. The data were analyzed using analysis of variance (F test) at 5% level, and if there were any real effects they were then followed by LSD test at 5% level.

Results and Discussion

Components of soybean plant growth

Based on the analysis of variance and LSD test at 5% level, the significant effect (Table 1) on plant height, number of leaves and leaf area was possibility because the applied microbes were capable in decomposing organic materials originated from waste of mushroom media (growing media) so that the nutrients required for plant growth could be provided. According to Sutedjo (1991), during the decomposition process, a number of materials/substances of microbial cells are fully integrated, which then materials/substances are broken down by various other microorganisms.

The nodules parameter also showed significant effect (Table 2). Nodules root was formed by association between the roots of soybean plants with *Rhizobium* bacteria. *Rhizobium* will fix N from the air to meet the needs of N for plant growth. According to Gardner et al. (1991), *Rhizobium* survival in nature is highly dependent on soil conditions, especially pH, moisture, organic matter, and the length of the distance between its host plant cultivation. If nodules are on nutrient-deficient soil conditions, they will be formed many nodules and actively fixed N from the air as shown in the number of formed nodules. Flowering age parameters showed significant effect (Table 2).

Results of observations showed that the treatment i_6 (EM4 applied 1 week after planting) made soybean plants begin to flower at age 39 DAP. According to Gardner et al. (1991), the soybean plants are sensitive to differences in the length of day, especially during formation of flowers. The process of flower formation is controlled by environmental factors, especially photo period and temperature, as well as by genetic or internal factors, particularly growth regulators, photosynthesis results, and supply of nutrients and minerals (e.g, nitrogen). At high temperatures and low humidity, the amount of sun light on the axillary stalk is more. This will stimulate the formation of flowers (Adisarwanto, 2006).

Component of soybean plant

Data presented in Table 3 show that in general the i_6 treatment (EM4 applied 1 week after planting) had good results in accordance with the emergence of flowers, allegedly because the applied microbes were capable of decomposing organic materials waste originatedfrom mushroom media (growing media) so that the nutrient required for plant growth could be provided which in turn further affected crop yield. According to Rinsema (1993), plants can thrive when the nutrients needed are available and in a form that can be absorbed by plants. The absorption of nutrients absorbed by plants is influenced by the availability of nutrients in the soil. Adequacy of nutrients will accelerate and enhance the growth of plants and then photosynthesis process also increase, which further will increase the result of photosynthesis assimilates number as well.

The amount of dumping products of photosynthesis will also affect the growth and plants production. According to Harjadi (1990), the manifestation of the reproductive phase require a supply of carbohydrates in the form of starches and sugars. If a plant growing flowers, fruits and seeds or storageorgans,not all carbohydrates are used for the development of the stems, leaves and roots. Most of the storages areremained for the development of flowers, fruits and seeds or tools inventory.

Results of analysis of variance and LSD test at 5% level indicated that there were significant effects on number of total pods/plant, pods contain, fresh weight of containing pods, dry weight of containing pods, dry weight of containing seed and weight of 100 seeds (Table3). The highest weight of 100 seed grains was produced by i_6 treatment (EM4 applied 1 week after planting) which was 8.74 g and which was significantly different from the I_0 (control). However, this was still less then description of soybean Wilis variety which equal to ± 10 g/100 seeds. More heavy seeds per 100 grams will increase production and better if the seeds used as seedling.

Large seed size gave higher total yield of dry beans. According to Goldworthy and Fisher (1996), the seed filling produced by photosinthate after flowering and translocation will be redeposited as photosinthate stored. According to Gardner et al. (1991), along with the reproductive growth period of crops that produced make seeds as dominant organ utilization (reserves a store of food and proliferation). Therefore, during seed filling, photosinthate newly formed or already stored can be used to increase the seed weight. Rusmiati et al. (2005) reported that not all the pods that were formed completely filled by seeds. This could be caused by a variety of disorders including unfavorable climatic conditions during flowering and presence of pests and plant diseases during pod filling.

Table 1. The mean of plant height (cm), number of leaves, leaf size (cm^2) as affected by inoculation and time of application of microbes (*Rhizobium* and EM4) in plants at different ages

Treatments	Plant Height (cm)			Number of Leaves			Leaf Size (cm^2)		
	Days After Planing (DAP)								
	50	**57**	**64**	**50**	**57**	**64**	**50**	**57**	**64**
i_0	47.42 ab	51.46 a	51.57 a	16.67 a	23.58 ab	28.42 ab	2.279.21 ab	2.828.24 b	2.894.90 b
i_1	46.63 a	51.40 a	53.04 a	19.83 bc	28.33 d	32.92 cd	2.856.54 b	3.232.21 b	3.235.54 b
i_2	49.38 abc	51.25 a	54.83 a	17.17 a	25.25 abc	30.17 bcd	2.350.75 b	2.560.97 ab	2.627.63 ab
i_3	51.26 bc	55.04 b	55.34 ab	16.92 a	25.33 abcd	31.25 bcd	2.504.09 b	3.086.45 b	3.086.45 b
i_4	46.00 a	50.54 a	51.54 a	17.92 abc	24.42 abc	29.42 bc	2.265.98 ab	2.844.31 b	2,877.64 b
i_5	45.87 a	50.13 a	54.42 a	20.25 c	27.00 cd	33.42 d	2.694.10 b	3.196.62 b	3.196.62 b
i_6	47.67 ab	53.75 b	55.30 ab	17.00 a	24.75 abc	31.00 bcd	2.841.03 b	3.139.15 b	3.142.48 b
i_7	44.95 a	46.63 a	51.56 a	16.67 a	22.33 a	25.67 a	1.737.81 a	1.732.18 a	1.765.51 a
i_8	52.54 c	56.38 b	59.67 b	18.58 abc	25.58 bcd	30.08 bcd	2.450.13 b	3.144.90 b	3.078.23 b
i_9	48.13 abc	52.25 b	53.63a	17.75 ab	24.67 abc	30.92 bcd	2.492.11 b	3.054.96 b	3.054.96 b
LSD 5%	4.53	4.95	4.40	2.45	3.02	3.72	609.25	845.45	829.23

Remarks: Numbers are coupled with the same letter in the same column show no significant difference in LSD test at 5% level. I_0: control (no treatment), i_1: *Rhizobium* 1 week application before planting, i_2: *Rhizobium* application at planting time, i_3: *Rhizobium* application 1 week after planting, i_4: EM4 1 Week application before planting, i_5: EM4 application at planting time, i_6: EM4 application 1 week after planting, i_7: *Rhizobium* and EM4 application 1 week before planting, i_8: *Rhizobium* and EM4 application at planting time, i_9: *Rhizobium* and EM4 1 Weeks application after planting.

Table 2. The mean of total root nodule, days to flowering, number of flowers, and total flower as affected by time application and microbial inoculation (*Rhizobium* and EM4) at various age of observations

Treatments	**The mean of total root nodule at various age of observations (weeks after planting)**				**The mean of days to flowering**	**The mean of totalthe number of flowers (florets)at various age of observations (days after planting)**			**Total amount of the number of flowers (florets)**
	4	**6**	**10**	**14**		**43**	**50**	**57**	
i_0	4.83 abc	18.00 bc	21.33 a	23.67 a	42.33 b	8.83 ab	65.99 cd	99.99 bc	154.54 a
i_1	7.67 cd	21.67 c	23.33 ab	27.67 abc	40.33 b	11.92 bc	65.68 cd	100.25 bc	177.85 d
i_2	3.33 a	8.67 a	26.67 bc	29.00 abcd	42.33 c	10.17 bc	66.67 d	90.42 ab	170.59 cd
i_3	3.17 a	17.33 bc	34.00 e	24.00 ab	42.33 c	10.50 bc	64.05 bcd	98.25 bc	172.80 cd
i_4	6.83 bcd	14.00 ab	31.33 de	24.67 ab	42.00 bc	9.83 bc	66.65 d	86.75 ab	163.23 abc
i_5	8.83 d	16.67 bc	22.67 ab	33.33 bcd	42.00 bc	12.33 c	65.19 cd	114.25 cd	168.44 bcd
i_6	4.67 ab	17.00 bc	28.67 cd	37.67 d	39.33 a	13.08 c	56.76 a	125.67 d	178.84 d
i_7	8.50 d	14.67 ab	24.67 abc	30.00 abcd	41.00 b	6.25 a	61.28 abc	73.67 a	157.87 ab
i_8	4.67 ab	12.00 ab	28.33 cd	28.00 abc	41.00 b	11.25 bc	67.13 d	91.75 ab	176.80 d
i_9	5.50 abc	17.67 bc	22.67 ab	36.67 cd	42.33 c	11.58 bc	59.89 ab	102.92 bc	174.39 cd
LSD 5%	2.93	6.44	4.07	9.50	0.93	3.25	4.78	19.93	11.64

Noted : Numbers are coupled with the same letter in the same column show no significant difference in LSD test at 5% level. WAP: Weeks After Planting, DAP: Days After Planting, I_0: control (no treatment), i_1: *Rhizobium* 1 Week aplication before planting, i_2: *Rhizobium* application at planting time, i_3: *Rhizobium* application 1 Week after planting, i_4: EM4 1 Week application before planting, i_5: EM4 application at planting time, i_6: EM4 application 1 Week after planting, i_7: *Rhizobium* and EM4 application 1 Week before planting, i_8: *Rhizobium* and EM4 application at planting time, i_9: *Rhizobium* and EM4 1 Weeks application after planting.

Table 3. The mean of Total Productive Branch / Plant, Total number of pods / plant, number of filled pods, Weights Dry-filled pods(g), Total weight of Dried Seeds (g), 100-seeds weight (g) and Dry weights of stover(g) at time of harvest due to treatment Inoculation and Time Application of Microbes (*Rhizobium* and EM4)

Treat ments	Number of productive branch / plant	Total number of pods / plants at harvest time	Number of soiled pods	Dry weight of soiled pods	Means of total weight of dried seeds (g)	Means of 100-seeds weight (g)	Means of dry weights of stover (g)
i_0	4.67 a	125.78 a	27.56 a	7.79 a	6.38 a	5.19 a	55.29
i_1	5.22 ab	152.89 bcd	37.22 ab	9.01 ab	7.50 cd	8.18 c	50.40
i_2	5.11 ab	151.44 abcd	32.56 ab	9.20 b	7.37 bcd	6.51 b	43.18
i_3	5.11 ab	165.89 cd	32.89 ab	8.71 ab	7.23 bcd	6.64 b	45.73
i_4	5.44 ab	139.56 abc	30.67 ab	8.57 ab	6.65 ab	6.46 b	32.73
i_5	5.56 ab	131.56 ab	32.00 ab	8.82 ab	7.60 cd	6.52 b	52.29
i_6	5.22 ab	158.67 cd	54.00 c	10.77 c	7.95 d	8.74 c	55.73
i_7	5.67 b	141.89 abc	40.44 b	9.29 b	6.44 a	5.71 ab	43.73
i_8	6.78 c	171.55 d	31.11 ab	9.72 bc	6.60 ab	8.49 c	58.62
i_9	5.22 ab	156.89 bcd	35.67 ab	9.55 bc	6.98 abc	8.55 c	45.06
LSD 5%	0.98	27.07	11.73	1.32	0.78	1.03	NS

Noted : Numbers are coupled with the same letter in the same column show no significant difference in LSD test at 5% level. NS: Not Significant, I_0: control (no treatment), i_1: *Rhizobium* 1 week application before planting, i_2: *Rhizobium* application at planting time, i_3: *Rhizobium* application 1 week after planting, i_4: EM4 1 Week application before planting, i_5: EM4 application at planting time, i_6: EM4 application 1 week after planting, i_7: *Rhizobium* and EM4 application 1 week before planting, i_8: *Rhizobium* and EM4 application at planting time, i_9: *Rhizobium* and EM4 1 Weeks application after planting.

Conclusion

In this research, we report the effects of microbial inoculation and the time of application (*Rhizobium* and *EM4*) on the growth and yield of soybean (*Glycine max* (L.) Merrill). The result could be summarized as follows: significant effect on the components of growth and yield, $i_6$treatment (EM4 which applied 1 week after planting) was the best treatmentcompared with controls as shown by the increasing on number of filled pods was 95.94%, dry weight of filled pods was 38.25%, total of dry weight seed was 24.61% and 100-seed weight was 68.40%.

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