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Research Article

Glomus mosseae AND *Pseudomonas fluorescens* AGAINST *Soybean Mosaic Virus* UNDER DRIP IRRIGATION SYSTEM

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ARTICLE HIGLIGHTS

- *Glomus mosseae* and *Pseudomonas fluorescens* enhance soybean resistance to SMV.
- Ten-day drip irrigation stop at bloom stage boosts seed yield and weight.
- Treatment reduces disease severity and increases *P. fluorescens* population.
- Water-saving irrigation method improves soybean performance in dry seasons.
- Combining *G. mosseae* and *P. fluorescens* increases soybean productivity.

Article Information

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ABSTRACT

Soybeans [*Glycine max* (L.) Merr.] require much water, especially in the early stages of growth, flowering, and pod formation and filling. Drought stress on soybeans will increase with global climate change. The research aimed to evaluate the efficacy of *Glomus mosseae* and *Pseudomonas fluorescens* in inducing systemic resistance to control *Soybean Mosaic Virus* (SMV) and the performance of soybean plants with drip irrigation in the dry season. The drip irrigation was stopped for ten days at the beginning of bloom, full bloom, beginning pod, and regular drip irrigation. The application of *G. mosseae* inoculants used 100 g of sterile compost mixed with 30 spores. The liquid inoculum of *P. fluorescens* was applied on cotyledonary leaves using the watering method. The plants were inoculated with SMV 6 and 12 days after the *P. fluorescens* treatment. The symptoms of SMV were recorded two weeks after inoculation, and Indirect ELISA detected the virus. The results showed the combination of *G. mosseae* and *P. fluorescens* under drip irrigation stopped at the beginning of bloom for ten days on ELISA absorbance values and disease severity of 0.259 and 3.72 %, respectively. Moreover, this treatment showed the highest values for the 100-seed weight, seed yield, and fresh weight of leaves. The technique of giving water by stopping drip irrigation at the beginning of bloom for ten days will help save water and increase the *P. fluorescens* population optimally in Alfisols with a pH of between 4.5 and 5.1 with incredibly low fertility. The combination of *G. mosseae* and *P. fluorescens* population resulted in a significant increase in the number of *G. mosseae*'s spores by stopping drip irrigation at the beginning of bloom for ten days.

Keywords:
agronomic performance, *glomus mosseae*, *pseudomonas fluorescens*, *soybean*, *soybean mosaic virus*

INTRODUCTION

Especially in the early stages of growth, flowering, and pod formation and filling, soybeans [*Glycine max* (L.) Merr.] require much water. Lack of water can inhibit the plants' growth, thereby reducing production yields. However, drought stress on soybeans will increase with global climate change. Nachappa *et al.* (2012) reported that drought stress reduced plants' disease resistance. In addition, drought stress and infection of viruses can decrease photosynthetic efficiency and the productivity of soybean crops. As a result, the soybean plant has problems with absorbing nitrogen, phosphorus, and potassium. In addition, dry land conditions cause low production and land use in the dry season. Drip irrigation is a very efficient method of giving irrigation water because the water is provided only in the root area. Therefore, it is very suitable to be applied on dry land.

In Indonesia, farmers often grow soybean plants on dry land with low fertility. Therefore, the plants' growth cannot be optimal. The *Soybean Mosaic Virus* (SMV) is one of the most common diseases and detrimentally impacts soybean production. The resistance of the new cultivar to the SMV requires organic matter with nutrients readily available to plants, such as elemental phosphate. However, fertilization with high doses of phosphates have caused susceptible plants. Therefore, biological fertilizers, such as mycorrhizae, are given to anticipate this situation. Mycorrhizal fungi can reduce drought stress and positively affect crop yields on dry land. Soybean yield losses due to drought stress are determined by the variety, duration of stress, and growing stadia (Rigano *et al*. 2016; Aminah *et al.* 2019). The application of mycorrhizae with seed coating is better than that in planting media, in which drought stress affects the development of mycorrhizae. Still, mycorrhizae cannot directly attach to the sources, so a material that can stick mycorrhizae to roots is needed. In addition, varieties, duration of drought stress, and growth stadia have affected soybean plants by inhibiting the seed-filling phase (Yooyongwech *et al*. 2019; Andayanie *et al.* 2019[a]). Moreover, Plant-Growth-Promoting Rhizobacteria (PGPR) are adaptive to or tolerant under drought-stress conditions. For example, Genus *Pseudomonas* is one of PGPR that may form symbiosis with the host plant and, therefore, may play a role as a biofertilizer and biocontrol agent. *Pseudomonas putida* KT2440 and *Pseudomonas fluorescens* are often employed for phosphorus fertilization to improve plant growth under drought stress and saline stress (Pirttilä *et al*. 2021; Costa-Gutierrez *et al*. 2021).

Pseudomonas spp. can increase plant biomass, relative moisture content, leaf water potential, soil/root tissue ratio attached to roots, aggregate stability, average weight diameter, and leaf water loss. Plant growth-promoting *Pseudomonas* spp. affected compatible solutes, antioxidant status, and plant growth of maize under drought stress. Inoculated plants have shown higher proline, sugar, and free amino acids. Seedlings inoculation with *Pseudomonas* spp. showed significantly lower activity of antioxidant enzymatic, ascorbate peroxidase (APX), catalase (CAT), and glutathione peroxidase (GPX) under drought stress. However, this fact indicates that maize seedlings experienced less stress than those not inoculated with *Pseudomonas* spp. Furthermore, the GAP-P45 strain exhibited the most significant influence on seedlings' growth biochemical and physiological status under drought stress (Sandhya *et al*. 2010; Meliani *et al.* 2017; Costa-Gutierrez *et al*. 2021). Late pod formation at the beginning of the full-pod stage to early seed fill was the most critical. The seed filling stage required much water and nutrients from the plant (Berglund *et al.* 1999).

Rhizosphere microbes and drip irrigation have prospects for eco-restoration in drought stress. *Pseudomonas* spp. is proved to be an alternative chemical fertilizer and supports Arbuscular Mycorrhizal Fungi (AMF) activity. AMF and *Pseudomonas* have synergistic potential. *Pseudomonas* dissolves phosphate, and AMF helps absorb phosphate in plants through AMF hyphae. AMF colonization with *Pseudomonas* could increase nutrients and water absorption (Gough *et al.* 2020; Sharma *et al*. 2021). Mycorrhizal fungi and *P. fluorescens* bacteria in the rhizosphere also improve soybean plants' nutrient transmission efficiency and stress tolerance in ecological restoration. Drip irrigation can overcome water shortages, irrigation depending only on rainwater, low soil fertility, shallow tillage layers, and low organic matter content. The drip irrigation method will help save water when water resources are minimal during the dry season. The optimal combination of Arbuscular Mycorrhizal Fungi (AMF) and *P. fluorescens* bacteria has a synergistic interaction to increase resistance under drought stress, resulting in high-yielding plants (Artursson *et al.* 2006). The restoration field provided an overview of the future application prospects in drought stress by rhizosphere microbial. Biological management of dry land uses the introduction of the beneficial microbe as a natural agent to increase the microbial population's quality of fertile soil. The research aimed to evaluate the efficacy of *G. mosseae* and *P. fluorescens* in inducing systemic resistance to control SMV and the performance of soybean plants undergoing drip irrigation in the dry season.

MATERIALS AND METHODS

Weather Condition at the Experimental Site

The experiments were conducted in the dry season during the drought stress in Gunung Kidul Regency, Yogyakarta, Indonesia, with an average temperature of 29.7 °C and 83.1% relative humidity (RH). The field experiments were initiated during the dry season from early May to July 2022. The average monthly rainfall in May, June, and July of 2022 was 24 mm, 21 mm, and 20 mm, respectively, representing monthly rainy days of 19, 22, 8, and 4 days, respectively.

Water Supply during the Dry Season

This research used the rainwater harvesting (RWH) system as the water supply in the drought stress area. The rainwater harvesting (RWH) system was made at a height of 5 meters, and the rainwater was placed in a tank with a capacity of 1,200 L. After the water was collected during rain, it flowed into the planting area through a one-inch diameter pipe. Horizontal branches were ¾ inch-plastic hoses, and the water flowed to each plant through a small hole.

Soybean Seed and Plant Preparation

Soybean seed preparation was done using seed priming in water (hydro priming) and salt solution (halo priming). Giving water or a solution to the seeds allows the seeds to survive in very arid field conditions, and they will usually germinate. Soybean seeds were obtained from the selection of lines resistant to the SMV (Andayanie *et al*. 2017; Andayanie *et al*. 2019[a]). The GK/L-T soybean lines were planted in 7 rows, 3 x 1.5 m^2, with a row spacing of 50 cm. The use of cashew nutshell extract prevented insect pests from landing and mosaic diseases (Andayanie & Ermawati 2019; Andayanie *et al*. 2019[b]; Andayanie *et al*.2019[c]).

P. fluorescens and *G. mosseae* were isolated from the soybean [*Glycine max* (L.) Merrill] rhizosphere plants under dry land in Gunung Kidul Regency, Yogyakarta, Indonesia. Soil samples required 20 sampling times, and each sampling weighed 10 kg. Phosphate solubilizing bacteria (PSB) assays used pure bacterial isolates grown on a solid Pikovskaya medium. The formation of a clear zone around the colony indicated the growth of PSB colonies. In addition, the index of phosphate-solubilizing bacteria was applied to potato tubers (Lelliot & Stead 1987). This test was aimed to examine the pectinolytic ability of bacterial isolates. The isolates reacted negatively and were used as potential isolate candidates. Liquid inoculum of *P. fluorescens* was inoculated on cotyledonary leaves seedlings (1 x 10^7 mL (CFU). *G. mosseae* were identified morphologically by Brundrett *et al.* (1996). This identification was based on morphological characteristics of *G. mosseae* and evaluated microscopically. The application of *G. mosseae* inoculants used 100 g of sterile compost mixed with 30 spores, and they were inserted into the holes around the roots. The holes were subsequently covered with soil again. The inocula of *G. mosseae* were applied seven Days After Planting (DAP).

Drip Irrigation Implementation

A water supply during the dry season was provided by constructing an iron frame tower with a height of 5 m, and a water reservoir with a capacity of 1,200 L is placed at the top. The water was collected during rain and then directed to the plants using a one-inch diameter pipe, which is further channeled through a ¾ inch diameter pipe to enhance water pressure. As a result, the gravity and the pump pressures have a drip rate of 0.62 L/hour and 1.15 L/hour, respectively. Water was supplied to each plant by making a small hole in the secondary hose (¾ inch), so that the water is directed only at the plant and not at the entire planting area.

Soybean Mosaic Virus Application

The SMV isolates were obtained from a collection of the Plant Protection Laboratory, Merdeka Madiun University, Indonesia. The inoculum was propagated in the soybean Wilis variety. One week and two weeks after inoculated with *P. fluorescens*, the plants were inoculated with SMV isolates using a compressed air technique at a pressure of 1.8-2.0 kg/cm^2, 1 g of inoculum from the infected leaves, 50 mL of buffer solution, 600 mesh Carborundum with 0.5% concentration (0.5 g/100 mL), and spray time 0.5 seconds/plant at a distance of 10 to 15 cm from the leaf surface of the plant.

Disease Severity and Detection of *Soybean Mosaic Virus*

The percentage of disease severity was calculated 60 days after SMV inoculation using the scale used by Andayanie *et al.* (2019) and Andayanie *et al.* (2022) as follows:

1 = healthy plants without visible symptoms on all leaves
2 = mild mosaic symptoms (10% to 30% leaf infection)
3 = moderate mosaic symptoms with green mosaic and smaller leaves (30% to 50% leaf infection)
4 = prominent mosaic symptoms with blistering and stunting (51% to 70% leaf infection)
5 = highly severe mosaic symptoms with stunting (> 71% leaf infection)

The scaling results were calculated using the following formula:

$$DS = \frac{\Sigma (n \times v)}{N \times V} \times 100\%$$

where:

DS = disease severity (%)
n = sum of infected leaves in each category
v = value scale of each category
N = total number of observed leaves
V = highest scale value

Serological detection was needed to see the absorbance ELISA values (AVE) and the reaction of the observation samples. Therefore, Indirect ELISA (I-ELISA) was used to detect the virus through utilizing SMV antisera. The I-ELISA was implemented using the modified Koenig (1981) method. A positive sample was indicated by a change in color in test wells, and the calculation of optical density (OD) values was done using an ELISA reader.

Statistical Data Analysis

The field data was analyzed on a split-plot in a Randomized Completely Block Design (RCBD) with two factors and four replicates. The first factors were drip irrigation treatments, which included stopping drip irrigation for ten days at the beginning of bloom, stopping drip irrigation for ten days at full bloom, stopping drip irrigation for ten days at the beginning pod, and regular drip irrigation carried out when the plants were 3-5 days old (germination phase), 15-20 days (vegetative stage), 25-35 days (flowering phase), and 55-70 days (pod filling phase). The second factors were the use of *P. fluorescens*, treatment with *G. mosseae*, the application of *P. fluorescens* combined with *G. mosseae*, and absence of microbial inocula application as the control. Eight weeks after planting, observation of the growth and development of the plants included (a) total root length, (b) root surface area, (c) number of root nodules, and (d) the plant height (cm). Eight weeks after planting, the observed dry weight of the leaf area included typical leaf weight, net assimilation rate, relative growth rate, and harvest index. Observations of yield and yield components included the number of pods, the number of seeds, the weight of seeds per plant, and the weight of 100 grains (g).

The data were analyzed using ANOVA and the statistical program SAS 9.1 computer package. The differences among treatment means were determined by Duncan's Multiple Range Test (DMRT) at $P < 0.05$.

RESULTS AND DISCUSSION

Initial Soil Chemical Properties

The initial soil chemical properties were collected from Gunung Kidul Regency, Yogyakarta, Indonesia. Table 1 shows that Alfisols have low organic carbon content. Therefore, adding manure as an essential fertilizer can overcome the low organic C content. Moreover, the acidity and physical properties of soil with high clay content were measured by the Bray-1 method. Phosphate compounds are essential for soybean growth. However, phosphorus (P) in the ground is bound by Al to form insoluble Al-P bonds, most of which cannot be absorb by plants (Hanyabui *et al*. 2020).

Table 1 Condition of soil chemical properties before experiments

Chemical properties	Value	Criterion
pH H2O	4.5-5.1	Acid
C-organic (%)	1.35-1.71	Low
N (%)	0.06-0.018	Very low
P1 Bray (ppm)	0.81-8.21	Low
K (me100/g)	0.23-0.55	Low-height

Source: Result of soil analysis conducted in the Physics and Chemistry Laboratory, Faculty of Agriculture of Universitas Gadjah Mada, Indonesia (2022).

The alternative that can be used to overcome the less productive land above is the use of the microbial rhizosphere. *P. fluorescens* bacteria that can dissolve phosphate are not available in a form that is available for plants to absorb it. Phosphorus transformation by solubilizing bacteria phosphate can increase phosphate availability in the soil. Vyas & Gulati (2009) noted that the secretion of bacterial organic acids such as acetic, formic, propionic, glycolic, lactic, glyoxylic, fumaric, succinic, citric acids, and tartaric cause dissolution processes.

Disease Severity

The severity of the disease varies with the following symptoms, i.e., mild mosaic, green mosaic with vein banding and chlorosis on the leaf margins, wrinkled veins with dark green bumps (blisters), mosaic with malformations in the form of curly and blistered leaves, stunted plants, and mosaic with cupping and stunted plants. The application of *G. mosseae* with *P. fluorescens* had the most extended incubation period and the appearance of presumably healthy symptoms, especially when drip irrigation was stopped for ten days at full bloom (Fig. 1; Table 2). However, applying *G. mosseae* in combination with *P. fluorescens* showed the lowest disease severity under regular drip irrigation. In addition, using these combined treatments could decrease the disease severity (Table 2).

Drought stress will affect the interaction between viruses and plants. For instance, soybean plants undergoing drip irrigation stopped for ten days at full bloom and the combination of *G. mosseae* and *P. fluorescens* will maintain the same incubation period for SMV symptoms as regular drip irrigation. As a result, symptoms of SMV decreases. In addition, the findings of Nachappa *et al.* (2016) showed that the response of plants to water stress would affect the composition of amino acids in the phloem and signaling pathways, impacting the development of aphids and viruses. Therefore, applying *G. mosseae* in combination with *P. fluorescens* in this study increased the production of secondary metabolites that played a role in inducing systemic resistance against SMV**.** Elsharkawy *et al.* (2012) and Shahzad *et al.* (2022) revealed that soybean plants treated with a treatment combining *G. mosseae* and endophytic Plant-Growth-Promoting Bacteria (ePGPB) had shown the plants' ability to act against viral diseases, including *Cucumber Mosaic Virus* and *Tobacco Mosaic Virus*.

Table 2 Disease severity and symptoms on soybean treated with *G. mosseae* and *P. fluorescens* under drip irrigation condition

Drip irrigation	**Disease severity/symptom (%)**			
	C	**Gm**	**Pf**	**Gm + Pf**
Regular drip irrigation	19.61/cl, gmv	8.63/Mb	7.83/mm,pph	3.68/pph
Stopping drip irrigation at the beginning of bloom for ten days	22.19/gmv, m	12.46/pph, cl	11.35/pph	9.08/cl, pph
Stopping drip irrigation at full bloom for ten days	19.94/ mc, wv	9.50/gm, cl	8.18/mm, pph	3.72/pph
Stopping drip irrigation at the beginning of the pod for ten days	26.22/mmb, mms	15.34/pph, Mb	15.91/gmv, mc	12.73/mc, mm

Notes: C = Control; Gm = *Glomus mosseae*; Pf = *Pseudomonas fluorescens*; Mb = mosaic with blotch; pph = presumably healthy (no symptom); gm = green mosaic, cl = chlorosis on the leaf margins; gmv = green mosaic with vein banding; mc = leaves malformation with curly; wv = wrinkled veins with dark green bumps (blisters); mmb = mosaic with malformations in the form of curly and blistered leaves; mms = mosaic with malformations in the form of curly and stunted plants; mc = mosaic with cupping and stunted plants.

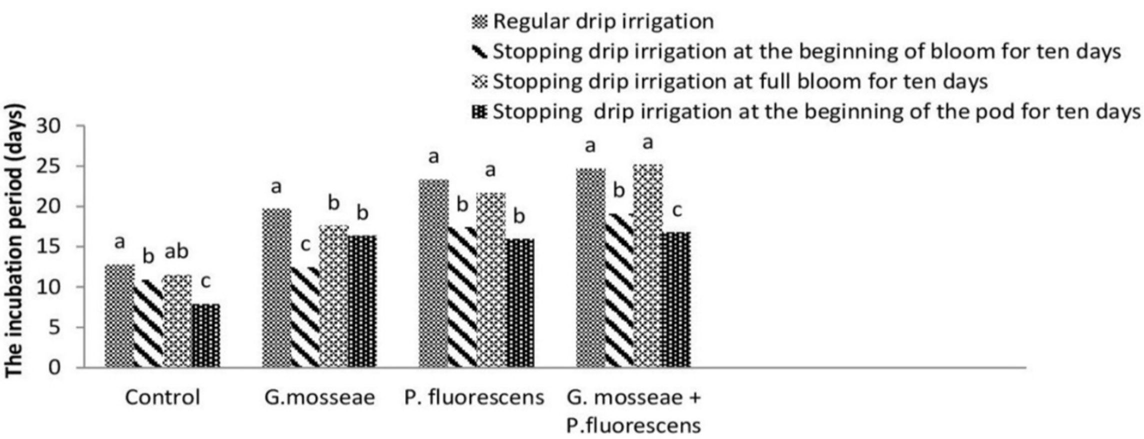

Figure 1 Comparing the incubation period means of SMV after microbial inoculation

Table 3 Effect of drip irrigation and microbial application on absorbance value of ELISA 60 days after SMV inoculation

Drip irrigation	ELISA absorbance value/reaction			
	C	Gm	Pf	Gm + Pf
Regular drip irrigation	2.569 abc/+	0.709 de/+	0.315 e/–	0.274 e/–
Stopping drip irrigation at the beginning of bloom for ten days	2.781 ab/+	1.853 abcd/+	1.561 bcde/+	1.336 cde/+
Stopping drip irrigation at full bloom for ten days	1.218 de/+	0.308 e/–	0.287 e/–	0.259 e/ –
Stopping drip irrigation at the beginning of the pod for ten days	2.989 a/+	0.787 de/+	0.741 de/+	0.312 e/ –

Notes: C = Control; Gm = *Glomus mosseae*; Pf = *Pseudomonas fluorescens*. Numbers followed by different letters in the same column show significantly different results based on Duncan's multiple intervals test at $\alpha = 0.05$. AVE negative control = 0.191; AVE positive control ELISA = 3.075. The test was declared positive if the AVE of the sample was twice the AVE of the negative ELISA control (positive if the AVE > 0.382). Values followed by the same letters indicate no significant differences based on Duncan's means comparison test ($P < 0.05$).

Table 4 Mean square values of soybean performance induced by microbial application under drip irrigation

SV	Df	PH	NFP	SW	SY	FWP
Replication	3	15.27	115.23*	175.42	0.91	171.68
Drip irrigation (a)	3	21.64	179.19*	72.30	47.36*	203.22
Microbial application (b)	3	43.51**	22.55	140.63	3.84	121.53*
Error (a)	9	18.70	24.68	46.91	7.62*	49.15
Error (b)	9	5.08	16.11	38.65	1.92	38.89
Drip irrigation x microbial application	9	36.92*	35.75	201.18*	3.01	61.77
Total	36	-	-	-	-	-
Coefficient of Variation (%)	–	9.13	13.69	10.68	15.88	20.12
SV	**Df**	**DWP**	**FWL**	**DWL**	**RL**	**NRN**
Replication	3	81.76*	32.61	0.91	0.43	93.21*
Drip irrigation (a)	3	23.15	167.98*	7.01*	10.65	39.37
Microbial application (b)	3	3.97	19.73	3.96*	25.18*	76.40*
Error (a)	9	65.46*	28.41	0.48	2.71	9.15
Error (b)	9	13.26	9.10	0.62	6.04	18.62
Drip irrigation x microbial application	9	24.46**	15.65	0.96	1.92	28.58
Total	36	-	-	-	-	-
Coefficient of Variation (%)	–	21.19	19.45	27.12	18.11	21.48

Notes: PH = plant height (cm); NFP = number of filled pods per plant; SW= the 100-seed weight (g); SY = seed yield (t/ha); FWP = fresh weight of plant (g); DWP = dry weight of plant (g); FWL = fresh weight of leaves (g); DWL = dry weight of leaves (g); RL = root length; NRN = number of root nodules/plant. Data were analyzed using analysis of variance (ANOVA) and the SAS 9.1 statistical program. The differences among treatment means were determined by Duncan's Multiple Range Test (DMRT) at $P < 0.05$.

By applying both *G. mosseae* and *P. fluorescens*, symptomatic plants could be detected using SMV antiserum, except for drip irrigation stopped for ten days at the beginning of bloom. Using either *G. mosseae* or *P. fluorescens* to all drip irrigation treatments inhibited SMV titers. However, combining *G. mosseae* and *P. fluorescens* in treatments also inhibited SMV titers Stopping drip irrigation at full bloom for ten days showed the lowest AVE. There was no significance in the data, and it reacted negatively with others, except with drip irrigation stopped for ten days at the beginning of bloom that responded positively to SMV antiserum (Table 3).

In the field test, the microbial application prevented stunted growth due to drought stress. However, drip irrigation and microbial application showed significant differences ($P \leq 0.05$) in the soybean growth stage and yield potential in the dry season (Table 2).

Soybean plants need water ranging from 360 mm to 405 mm from their growing period to harvest. In addition, the flowering phase until the pods formation requires 5.08 mm to 7.62 mm of water per day, and the ripening stage of the pods ready for harvest requires 1.27 mm to 5.08 mm of water per day (Mustikawati *et al*. 2018). The provision of water through drip irrigation and microbial application affected water availability in the soil for soybean plants, which root systems are shallow and very responsive to water availability. However, water availability below field capacity generally inhibits plant metabolism, affecting growth, development, membrane integrity, osmotic pressure, and crop yields. *G. mosseae* combined with *P. fluorescens* can be used to induce resistance to SMV using regular drip irrigation, drip irrigation stopped for ten days at full bloom, and drip irrigation stopped for ten days at the beginning of the pod formation.

Table 5 reveals that the plants' height of the GK/L-T soybean line undergoing drip irrigation stopped for ten days at the beginning of bloom had the lowest plant height (76.62 cm). There was the highest sensitivity to drip irrigation treatments, and there was no significant difference (P < 0.05) from planting with drip irrigation stopped for ten days (78.31 cm) at full bloom. The regular irrigation treatment showed the highest performance of soybean among test treatments. Stopping drip irrigation at full bloom for ten days produced more filled pods per plant than stopping drip irrigation at the beginning bloom for ten days and stopping drip irrigation at the beginning of the pod for ten days.

Soil nutrients, water availability, and sunlight influenced filled pod formation. Stopping drip irrigation for ten days at the beginning of pods formation caused pod loss and reduced the rate of assimilation transport into the pods, so that the number of seed pods was fewer than that of others. Bennett *et al.* (2011) noted that lack of water at pod formation caused the number of pods to form, and pod filling was not optimal. There were the most critical stadia. Data on seed yield showed no significant difference (P < 0.05) between stopping drip irrigation at full bloom for ten days and stopping drip irrigation at the beginning pod for ten days. Comparing the mean of the performance of soybean with *P. fluorescens* Pf-19 showed the highest plant height (PH), the 100-seed weight (SW), and the number of root nodules per plant (NRN) with an average of 58.14 cm, 15.13 g, and 45.21 per plant, respectively. There were no significant differences in the probability level of 5% with *Glomus mosseae* application (Table 6).

Table 5 Comparing the means of soybeans performance under drip irrigation

Drip irrigation	**PH**	**NFP**	**100 SW**	**SW**	**FWP**
Regular drip irrigation	93.11[a]	95.74[a]	21.03[a]	2.46[a]	37.23[a]
Stopping drip irrigation at the beginning of bloom for ten days	76.62[c]	52.30[c]	13.29[b]	2.01[b]	28.42[c]
Stopping drip irrigation at full bloom for ten days	78.31[c]	76.55[b]	20.47[a]	2.38[a]	34.06[b]
Stopping drip irrigation at the beginning of the pod for ten days	89.60[b]	43.18[d]	15.36[b]	1.97[b]	35.81[a]
Drip irrigation	**DWP**	**FWL**	**DWL**	**RL**	**NRN**
Regular drip irrigation	9.16[a]	18.71[a]	5.28[a]	26.14[a]	6.58[a]
Stopping drip irrigation at the beginning of bloom for ten days	6.32[c]	12.43[c]	3.65[b]	18.50[c]	3.76[b]
Stopping drip irrigation at full bloom for ten days	8.52[b]	17.16[b]	4.01[ab]	21.36[b]	3.81[b]
Stopping drip irrigation at the beginning of the pod for ten days	8.94[ab]	18.32[a]	4.97[a]	25.72[a]	5.92[ab]

Notes : PH = plant height (cm) ; NFP= number of filled pods per plant; SW= the 100-seeds weight (g); SW= seeds weight (t/ha); FWP= fresh weight of plant (g); DWP= dry weight of plant (g); FWL= fresh weight of leaves (g); DWL= dry weight of leaves (g); RL= root length (cm); NRN: number of root nodules. Values followed by the same letters indicate no significant difference based on Duncan's means comparison test (P < 0.05).

Table 6 Comparing the mean of soybean performance with microbial application

Microbial application	PH	NFP	SW	SY	FWP
Control	24.75c	4.05 c	9.92	1.72b	10.55b
Glomus mosseae	56.92a	9.24 a	15.13a	1.97a	13.21a
Pseudomonas fluorescens	58.14a	7.18 b	14.80a	1.85ab	10.76b
G. *mosseae* + *P. fluorescens*	41.05b	6.93 b	15.78a	2.01a	12.93a
Microbial application	**DWP**	**FWL**	**DWL**	**RL**	**NRN**
Control	3.11c	13.67b	3.25c	6.98b	25.95c
Glomus mosseae	4.63a	14.05a	4.38a	7.68a	45.21a
Pseudomonas fluorescens	3.52b	13.86b	3.60b	7.23ab	43.70a
G. *mosseae* + *P.fluorescens*	4.37ab	14.49a	3.81b	7.54a	36.86b

Notes: PH = plant height (cm); NB = number of branches per plant; NFP = number of filled pod per plant; SW = the 100-seed weight (g); SY = seed yield (t/ha); FWP = fresh weight of plant (g); DWP = dry weight of plant (g); FWL = fresh weight of leaves (g); DWL = dry weight of leaves (g); RL = root length; NRN = number of root nodules per plant. Values followed by the same letters indicate no significant differences based on Duncan's means comparison test (P < 0.05).

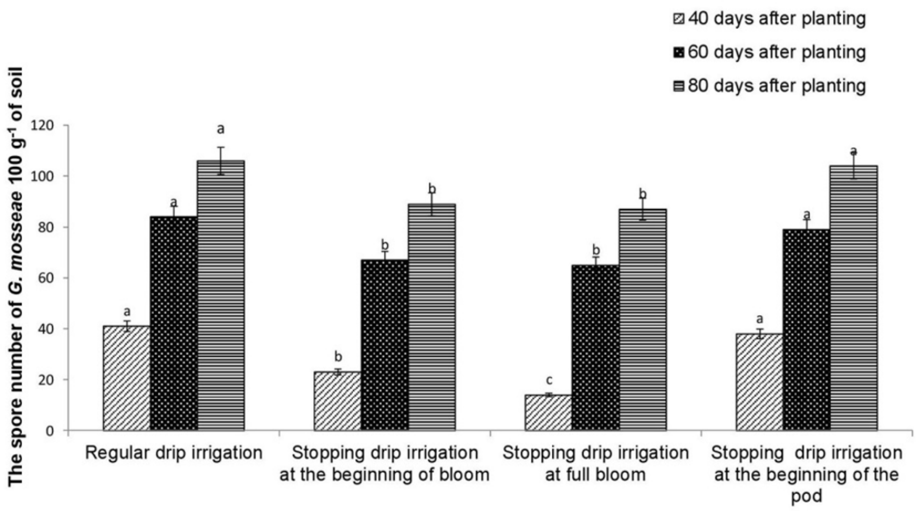

Figure 2 Mean value of the number of spores when inoculated with *G. mosseae* in soil

Spores Density of *Glomus mosseae*

Treatment of stopping drip irrigation at full bloom for ten days had the lowest mean value on the number of *G. mosseae* spores in soil, i.e., 14 spores at 40 days after planting (DAP). Moreover, *G. mosseae* spore development tended to increase at 60 DAP and 80 DAP. Gholamhoseini *et al.* (2013) noted that drought stress inhibited mycorrhizal colonization. However, *G. mosseae* is more efficient on sunflower plants under drought stress. However, mycorrhizal hyphae can still absorb water from the soil pores when plant roots experience difficulty. On the other hand, the regular drip irrigation treatment had the highest mean value of the number of spores at 40 DAP to 60 DAP. However, there were no significant differences in the number of spores under drip irrigation stopped for ten days at the beginning of the pod, which were 38, 69, and 104 spores in 100 g^{-1} of soil (Figure 2).

Pseudomonas fluorescens Population

The technique of giving water by stopping drip irrigation at the beginning of bloom for ten days can help save water and increase *P. fluorescens* population optimally on marginal soils with extremely low fertility, especially on P1 Bray (≤ 8.21ppm), N (≤ 0.018%), and pH (≤ 5.1). Therefore, stopping drip irrigation at the beginning of bloom for ten days can increase plant P uptake. Drip irrigation can significantly influence the spread rate of *P. fluorescens*. The lowest rate was in the regular drip irrigation. Stopping drip irrigation when soybean plants are very susceptible to water shortage showed a similar higher population of *P. fluorescens* in soil (Figure 3). However, discontinuing drip irrigation can reduce soil moisture content levels, so the soil lacks water and its cavity contains a considerable amount of air.

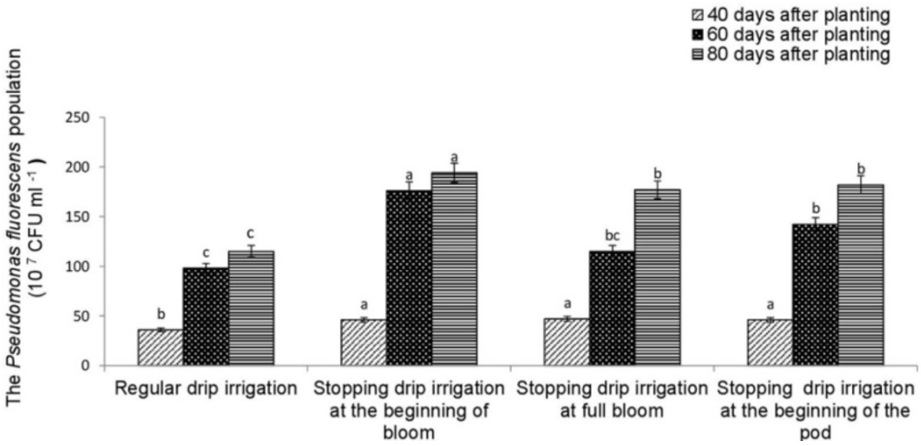

Figure 3 Mean value of the population when inoculated with *P. fluorescens* in soil

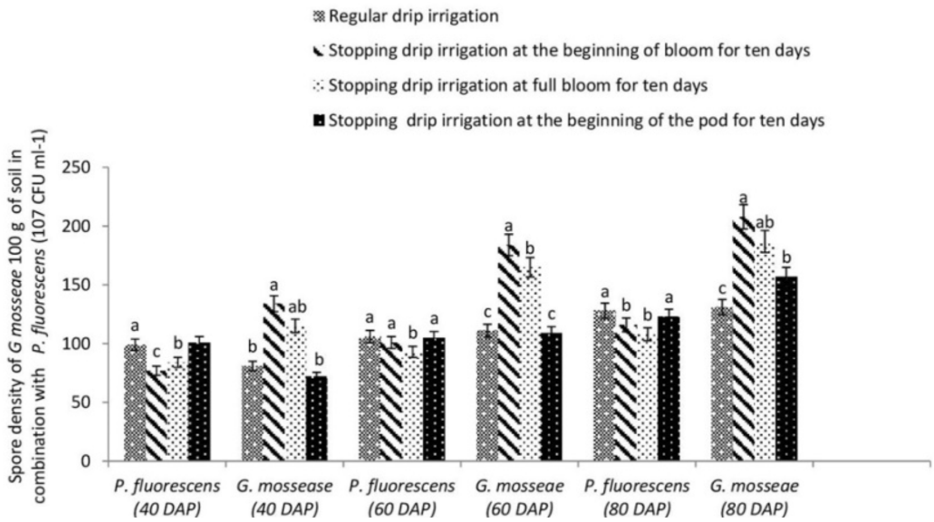

Figure 4 Mean value of the number of *G. mosseae*'s spores in combination with *P. fluorescens* population

Combination of *Glomus mosseae* and *Pseudomonas fluorescens*

The mean value of spores' density of *G. mosseae* in combination with *P. fluorescens* population presented in Figure 4 shows a significant increase in the number of spores of *G. mosseae* resulting from stopping drip irrigation at the beginning of bloom for ten days. *P. fluorescens* was also assumed to be the phosphate solubilizing microorganism, promoting a higher number of *G. mosseae* spores per 100 g soil.

Water content conditions in soils could inhibit the life of *Glomus* and *Pseudomonas*. Macro and micronutrient compounds were less effectively utilized for seed formation and yield. However, stopping drip irrigation at full bloom for ten days affected AMF and *Pseudomonas.* There were increased populations of *G. globulus* and *P. fluorescens* in the soil. The increase in plant dry weight reflects the number of nutrients absorbed and photosynthesis results. The higher the dry weight of a plant, the more nutrients are needed, and the plant's rate of photosynthesis is also high. Zhang *et al.* (2016) showed that drought conditions increased the CO_2 partial pressure among cells, so the photosynthetic performance of soybean plants decreased. Stopping drip irrigation for ten days at the beginning of bloom significantly reduced biomass production. The number of leaves and their size were small, causing a small amount of photosynthetic products (< 0.05). By comparing the mean, it is learned that stopping drip irrigation at the beginning of bloom for ten days was more sensitive to absorbing nutrients in photosynthesis. The low absorption of nutrients in plants disrupted the formation of chlorophyll and decreased the chlorophyll content in the leaves. This problem caused a decrease in the rate of photosynthesis and assimilation.

Regular drip irrigation and drip irrigation stopped for ten days at the beginning of the pod helped plants absorb more water and nutrients than drip irrigation stopped for ten days at the beginning of bloom and drip irrigation stopped for ten days at full bloom. The availability of water affected the fixing of nitrogen in the soil. Therefore, it influenced carbohydrate formation and increased the number of root nodules. In addition, the low content of C-organic (1.35% to 1.71%) and P1 Bray (0.81 ppm to 8.21 ppm) would stimulate mycorrhizal propagules to colonize the roots.

P. fluorescens application contributed to the highest plant height, and there was no significant difference from *G. mosseae*. Indol acetate acid (IAA) of *P. fluorescens* is essential in cell division and elongation. In addition, *P. fluorescens* solvent can dissolve phosphate still trapped in the soil, such as Fe, Al, Ca, and Mg elements into elements available for plants (Meliani *et al.* 2017). Meanwhile, *G. mosseae* can stimulate plant growth hormones, such as cytokinins and auxins. In addition, mycorrhizae's external hyphae can help the root system to absorb minerals, nutrients, and water more efficiently from the soil, especially in dry conditions. Other studies by Hanyabui *et al.* (2020) showed that mycorrhizal fungi can increase nutrient uptake, especially phosphorus, reduce abiotic stress, such as resistance to drought stress, and increase growth booster hormones. On the other hand, Pirttilä *et al.* (2021) and Costa-Gutierrez *et al.* (2021) noted that phosphate-solubilizing bacteria (PSB) could also improve the availability of P in acid soils. *P. fluorescens* is often used for phosphorus fertilization to enhance plant growth under drought and saline stress. The combination of *G. mosseae* and *P. fluorescens* under drip irrigation showed the highest increase in leaves' 100-seed weight, seed yield, and fresh weight. However, there was no significant difference ($p < 0.05$) in the effectiveness of *G. mosseae*. The application of *G. mosseae* could improve soil conditions and support nutrient absorption. The continuity of symbiosis between plants and *G. mosseae* will influence plant metabolic processes and the formation of new roots with high membrane permeability, which is advantageous for mycorrhizae's root colonization. Also, the wide distribution of hyphae in the soil allows plants to absorb more groundwater. Allen (2007) and Juyal (2021) noted that the drying patterns, the soil pore structure, the amount of hyphal elongation, and the spread rate of fungal hyphae in soil could play a key role in mycorrhizae in plant-water relations.

Bacteria spread through soil without any water movement. Soil physical conditions and architecture also affect the spread rate of bacteria through soil. For example, soil with lower bulk density facilitates faster spread of bacteria compared with soil with higher bulk density. Neetu *et al.* (2012) observed the secondary metabolite of *P. fluorescens* to increase the number of *G. mosseae*'s spores in soil. Furthermore, the study also indicated a significant increase in the absorption of nutrients and water in unfavorable conditions. The reason could be that *P. fluorescens* stimulates phosphate solubilization to increase the number of spores. However, the high phosphate solubilization levels could suppress the number of *G. mosseae*'s spores by stopping drip irrigation at the beginning of bloom for ten days.

CONCLUSION

Providing water with drip irrigation and microbial application can affect water availability in the soil for soybean plants. The technique of giving water by stopping drip irrigation at the beginning of bloom for ten days will help save water and optimally increase the *P. fluorescens* population in marginal soils with very low fertility.

REFERENCES

Andayanie WR, Santosa V, Rahayu M. 2017. Resistance to *Soybean mosaic virus* with high yield on F7 soybean lines. Int J Agric Biol 19(2):226-32.

Andayanie WR, Adinurani PG, Nuriana W, Ermawaty N. 2019a. The plant defence inducer activity of *Anacardium occidentale* Linn., *Azadirachta indica* A. Juss. and *Zingiber officinale* Rosc. extracts against *Cowpea mild mottle virus* infecting soybean. In: Arutanti O, Randy A, Fitriadi MA (Editors.). Proceedings of the 5th International Symposium on Applied Chemistry. AIP Conf. Proc. 2019. p. 020033-1–020033-8. Research Center for Chemistry Indonesian Institute of Sciences.

Andayanie WR, Ermawati N. 2019. Developmental effect of cashew nut shell extract against nymphal instar of silver leaf whitefly (*Bemisia tabaci* Genn.). Proceeding of the 2nd International Conference on Natural Resources and Life Science. IOP Conf Proc 2019 (293). p.1-7.

Andayanie WR, Nuriana W, Ermawati N. 2019b. Bioactive compounds and their antifeedant activity of cashew nut (*Anacardium occidentale* L.) shell extract against *Bemisia tabaci*, (Gennadius, 1889) (Hemiptera: Aleyrodidae). Acta Agric Slov 113(2):281-8.

Andayanie WR, Nuriana W, Ermawati N. 2019c. Antiviral activity of cashew nut shell extract against *Cowpea mild mottle virus* on soybean. J Trop Plant Pests Dis 19(2):170-8.

Artursson V, Finlay RD, Jansson JK. 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ Microbiol 8:1-10.

Bennett EJ, Roberts JA, Wagstaff C. 2011. The role of the pod in seed development: strategies for manipulating yield. New Phytol 190:838-53.

Berglund, Williams Mc, Endres GJ. 1999. Soybean Growth and Management Quick Guide. Revised November 2021 by Endres G, Kandel H (Editors). North Dakota State University. https://www.ndsu.edu/agriculture/ag-hub/publications/soybean-growth-and-management-quick-guide

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N. 1996. Working with Mycorrhizas in Forestry and Agriculture. Canberra (AU): AClAR Monograph.

Costa-Gutierrez SB, Caram-Di Santo MCV, Zenoff AM, Espinosa-Urgel M, Ezequiel de Cristóbal, R, Vincent P.A. 2021. Isolation of *Pseudomonas* strains with potential for protection of soybean plants against saline stress. Agronomy 11(2236):1-16.

Elsharkawy MM, Shimizu M, Takahashi H, Hyakumachi M. 2012 The plant growth-promoting fungus *Fusarium equiseti* and the *Arbuscular mycorrhizal* fungus *Glomus mosseae* induce systemic resistance against *Cucumber Mosaic Virus* in cucumber plants. Plant Soil 361:397-409. DOI :10.1007/s11104-012-1255-y

Gholamhoseini M, Ghalavand A, Dolatabadian A, Jamshidi E, Joghan AK. 2013. Effects of *Arbuscular mycorrhizal* inoculation on growth, yield, nutrient uptake and irrigation water productivity of sunflowers grown under drought stress. Agric Water Manage 117:106-14. DOI: 10.1016/j.agwat.2012.11.007

Gough EC, Owen KJ, Zwart RS, Thompson JP. 2020. A systematic review of the effects of *Arbuscular mycorrhizal* fungi on root-lesion nematodes, *Pratylenchus* spp. Front Plant Sci 11(923):1-14.

Hanyabui E, Apori SO, Atiah K, Abindaw T, Ali M, Asiamah JY, Byalebeka J. 2020. Phosphorus sorption in tropical soils. AIMS Agric Food 5 (4):599-616.

He DC, He MH, Amalin MD, Liu W, Alvindia DG, Zhan J. 2021. Biological control of plant diseases: An evolutionary and eco-economic consideration. Pathogens 10(10):1311. DOI: 10.3390/pathogens10101311.

Juyal A, Otten W, Baveye PC, Eickhors T. 2021. Influence of soil structure on the spread of *Pseudomonas fluorescens* in soil at microscale. Eur J Soil Sci 72:141-53.

Lelliot RA, Stead DE. 1987. Methods for the Diagnosis of Bacterial Diseases of Plants. Methods in Plant Pathology, British Society for Plant Pathology. London (UK): Blackwell Scientific Publications. 212 p.

Meliani A, Bensoltane A, Benidire L, Oufdou K. 2017. Plant Growth-Promotion and IAA Secretion wiith *Pseudomonas fluorescens* and *Pseudomonas putida*. Research & Reviews: J Bot Sci 6(2):16-24.

Mustikawati DR, Mulyanti N, Arief RW. 2018. Productivity of Soybean on Different Agroecosystems. Int J Environ Agric Biotech 3(4):1154-9.

Nachappa P, Culkin CT, Saya PM, Han J, Nalam VJ. 2016. Water stress modulates soybean aphid performance, feeding behavior, and virus transmission in soybean. Front Plant Sci 7(552):1-15. DOI: 10.3389/fpls.2016.00552

Neetu N, Aggarwa A, Tanwar A, Alpa A. 2012. Influencer of Arbuscular Mycorrhizal Fungi and *Pseudomonas fluorescens* at different superphosphate levels on linseed (*Linum usitatissimum* L.) growth response. Chilean J Agric Res 72(2):237-43.

Pirttilä AM, Tabas HMP, Baruah N, Koskimäki JJ. 2021. Biofertilizers and biocontrol agents for agriculture: How to identify and develop new potent microbial strains and traits. Microorganisms 9(817):1-17.

Rigano MM, Arena C, Di Matteo A, Sellitto S, Frusciante L, Barone A 2016. Eco-physiological response to water stress of drought tolerant and drought-sensitive tomato genotypes. Plant Biosyst 150:682-91.

Sandhya V, Ali SZ, Grover M. 2010. Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. Plant Growth Regul 62**:**21-30.

Shahzad GR, Passera A, Maldera G, Casati P, Marcello I, Bianco PA. 2022. Biocontrol potential of endophytic Plant-Growth-Promoting Bacteria against phytopathogenic viruses: Molecular interaction with the host plant and comparison with chitosan. Int J Mol Sci 23(6990):1-20. DOI: 10.3390/ijms23136990

Sharma M, Saini I, Kaushik P, Aldawsari MM, Balawi T, Pravej AP. 2021. Mycorrhizal fungi and *Pseudomonas fluorescens* application reduces root-knot nematode (*Meloidogyne javanica*) infestation in eggplant. Saudi J Biol Sci 28:3685-91.

Takahashi H, Fukuhara T, Kitazawa H, Kormelink R. 2019. Virus latency and the impact on plants. Front Microbiol 10(2764):1-18. DOI: 10.3389/fmicb.2019.02764

Yooyongwech S, Cha-Um S, Tisarum R, Terawitaya C, Samphumphung T, Aumtong S, …, Phisalaphong M. 2019. Influence of different encapsulation types of *Arbuscular mycorrhizal* fungi on physiological adaptation and growth promotion of maize (*Zea mays* L.) subjected to water deficit. Not Bot Horti Agrobo 47(1):213-20.

Zhang J, Liu J, Yang C, Dud S, Yang W. 2016. Photosynthetic performance of soybean plants to water deficit under high and low light intensity. South African J Bot 105:279-87.