# THE EFFECTS OF RHIZOBIUM AND ARBUSCULAR MYCORRHIZA FUNGI ON GROWTH AND NUTRIENT UPTAKE OF COWPEA VARIETIES

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## Abstract

The study was conducted to determine the effects of rhizobium and Arbuscular mycorrhiza fungi (AMF) on the growth of three cowpea varieties (*Vigna unguiculata* L. Walp.). The cowpea inoculated with three *Rhizobium* strains (R14, R41 and R55) and four AMF strains (AMF1, AMF2, AMF3 and AMF4) were evaluated with a pot experiment under greenhouse conditions. The experiment consisted of 3 groups and each group was divided into six treatments in completely randomized design: (1) control treatment (non-inoculated plant), (2) rhizobium treatment (inoculated with rhizobium isolated R14, R41 and R55 for cowpea no. 14, 41, and 55, respectively), (3) AMF1 treatment (dual inoculation with each rhizobium and AMF1), (4) AMF2 treatment (dual inoculation with each rhizobium and AMF2), (5) AMF3 treatment (dual inoculation with each rhizobium and AMF3) and (6) AMF4 treatment (dual inoculation with each rhizobium and AMF4). The results showed that rhizobium inoculation increased shoot dry weight of all cowpea varieties higher than control treatment with 14, 45, and 28%, respectively. The cowpea

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no.14 and 41 that rhizobium inoculation increased the amounts of nitrogen from nitrogen-fixation higher than control treatment by 70 and 47%, respectiv ely. The dual inoculation of rhizobium and AMF on a percentage of mycorrhizal colonization and phosphorus content were not statistically significant from the control treatment. The amounts of nitrogen from nitrogen-fixation of cowpea strain 14 and 55 with dual inoculation of *Rhizobium* R14 or R15 and all groups of AMF were lower than a single rhizobium inoculation. The amounts of nitrogen from nitrogen-fixation of cowpea strain 41 and 55 with dual inoculation of rhizobium R14 and AMF2 or AMF3 or AMF4 were higher than a single rhizobium inoculation. This study concluded that the growth of cowpea and amounts of nitrogen from nitrogen-fixation of cowpea could have been improved by specific rhizobium. However, the dual inoculation of rhizobium and arbuscular mycorrhizal remains to be considered on a case-by-case basis.

**Keywords:** cowpea, rhizobium, arbuscular mycorrhiza fungi, nitrogen fixation

## Introduction

Cowpea (*Vigna unguiculata* (L.) Walp var. *unguiculate*) is one of the most ancient crops known to humankind and is the most important legumes in Africa (Murdock et al., 2010). It has been extensively grown traditionally, which has grown to intercrop with sorghum, millet, maize, or cassava. However, it has grown as a monoculture on occasion. Farmers have lately increased their cowpea production after recognizing it could be a profitable crop. Cowpea grows in poor soil and low moisture (average annual rainfall of 300 mm). For the African continent, cowpea is an economic crop used as food and feed and more than 80% of the world’s cowpea crop is grown in Africa. Furthermore, the cowpea grain contains 23-25 % high-quality protein by weight, which is comparable to the quantity found in dried leaves (Murdock et al., 2010). In the field, cowpea is an N-source for plants and soil and also enhances soil fertility and protects soil moisture (Bado et al., 2006).

In Northern Thailand, *V. unguiculata* var. *unguiculate* is grown in dryland farming or garden at highland areas. For the selection of variety, three varieties of *V. unguiculata* were selected by Wongwai et al. (Wongwai et al., 2017) after they found that only cowpea can grow with upland rice not disturb rice. The three varieties were identified by color include No. 14 (black seed), No.41 (brown polka dot dotted color), and No.55 (black and white seed). Moreover, *V. unguiculata* var. *unguiculate* can protect soil moisture surface and reduce weed (Wongwai et al., 2014). The litter of cowpea can increase organic matter. The cowpea's pod can be food and sell (Wongwai et al., 2014).

Rhizobia are a type of soil bacteria that form root nodules in a symbiotic interaction with legume roots. They are a type that coexists with plants and can be utilized in environmental agriculture to reduce nitrogen fertilizer consumption, aid drought resistance, reduce nitrogen pollution and soil acidification, raise Leguminosae productivity and quality, and improve soil fertility and health (Young, 2014).

For cowpea, *Bradyrhizobium* strain has been increased in shoot biomass (Püschel et al., 2017). A beneficial microorganism is arbuscular mycorrhiza fungi (AMF) that facilitate enhanced plant phosphorus uptake (Klabi et al., 2014). However, depending on the degree of physiological compatibility between the fungal symbiont and the host plant, nutrient availability, and plant species relationships, the influence of AMF on the plant might be negative, neutral, or beneficial (Meng et al., 2015). The tripartite symbiosis between legumes, rhizobia, and mycorrhiza fungi is beneficial for legume nitrogen uptake (Estefan et al., 2013). The dual inoculation showed a significantly increased total biomass of soybean and maize compared with non-inoculated. Püschel et al.( 2017) found that *Bradyrhizobium strain* could be related significantly to the indigenous AMF, and showed a more various colonization pattern of cowpea root. However, rhizobium and arbuscular mycorrhiza's ability and potential on cowpea growth have not been investigated in Thailand. Therefore, our study aims to determine the effects of rhizobium and arbuscular mycorrhiza fungi (AMF) on cowpea and assess the capacities of the three rhizobium on cowpea growth.

## Materials and methods

### Experimental Design

A greenhouse pot experiment was conducted at the farm of Maejo University, Thailand. Wongwai et al. (2017) selected three cowpea varieties and separated the test as 3 experiments. Varieties were included as cowpea No. 14 (black seed), No.41 (brown polka dot dotted color) and No. 55(black and white seed). The experiment was conducted in six treatments, and four replicates with a completely randomized design. Each variety was treated with compatible rhizobium. There were as follow: (1) only cowpea and no inoculant (control, T1); (2) cowpea inoculated with rhizobia isolate R14, R41, and R55, which selected by soil science laboratory in Maejo University (T2); (3-5) each cowpea varieties inoculated with rhizobia R14, R41, and R55 plus with arbuscular mycorrhiza fungi selected from Samoeng Rice Research Center (AMF1-AMF3) and from Department of Agriculture; DOA (AMF4) (6) each cowpea varieties inoculated with rhizobia isolated R14, R41 and R55 plus with arbuscular mycorrhiza fungi from Department of Agriculture (DOA). Transparency plastic greenhouse was used from April 24 – August 8, 2018. The harvesting was finished when cowpea was as 105 days after planting.

### Plant Growth Medium

The using soil contained pH 5.73, 3.8 g $kg^{-1}$ of organic matter, 43 ug $g^{-1}$ of available P, and 147 ug $g^{-1}$ of exchangeable K. The soil was twice steamsterilized at 120° C for 2 hours to eliminate the AMF spores. Next, plastic pots having 30 cm diameter and 27 cm height were filled with 8 kg of dry soil.

**Seedling and Inoculating**

A total of 3 seeds of each cowpea varieties (*V. unguiculata* var. unguiculate Varieties No. 14, No.41 and No. 55) were sterilized in 70% ethyl alcohol for 5 min. The sterilized cowpea seed was covered with peat moss which was $5.9\times10^{11}$ cfu $g^{-1}$ of rhizobium. Seeds were sowed in the tray and 13 days old seedling was transplanted to a pot. For arbuscular mycorrhiza inoculation, cowpea seedlings were planted to the pot after 50 g of soil inoculum (contained 15-20 spores $g^{-1}$) were added to 8 kg of soil $pot^{-1}$. Cowpea were grown under greenhouse, and watering was every 1-2 day depend on the weather. Two weeks after inoculations, the seedlings were thinned to one seedling per pot.

**Data collection**

After 105 days after planting, dry weight of shoot dry weight, root dry weight, nodule dry weight, and branches number of plant, nitrogen, phosphorus, potassium uptake, ureide relative index, mycorrhizal colonization and correlation coefficient between shoot dry weight (SDW) were analyzed. Determinations of nitrogen, phosphorus and potassium content of shoot: nitrogen content was determined by the Kjeldahl method as described by Estefan et al. (Herridge & Peoples, 2002). Phosphorus and total potassium were determined by the procedure of Estefan et al. (2013). Ureide-N, amino-N and nitrate-N, ureide relative index (RU-N%) was determined by Herridge & Peoples (2002). Mycorrhizal infection was used by Phillips & Hayman (1970) (Tang et al., 2019). Correlation coefficient between shoot dry weight(SDW) and All parameters were analyzed for statistical significance using the analysis of variance (ANOVA) by Sirichai Statistic program ver. 7.0.

## Results and discussion

The influence of rhizobium and arbuscular mycorrhiza fungi on the growth of three cowpea cultivars was investigated. The present research showed an encouraging effect of single rhizobia inoculation and dual inoculation with arbuscular mycorrhiza fungi (AMF) can increased dry shoot weight of cowpea, which is in agreement with white clovers after inoculated with AMF *Rhizoglomus intraradices* and *Rhizobium trifolii* (Boddey et al., 2016). Meng et al. (2015) also reported that the soybean and maize intercropping system's yield was signifi cantly increased by the single AMF *Glomus mosseae* or *Rhizobium* SH212 and dual inoculating. Our results also supported that some advantageous microbes' strains had to the synergistic work in stimulating the growth of host plant

**1. Cowpea no. 14**

**Shoot dry weight, nitrogen, phosphorus and potassium uptake and their relative:** The comparison of the control treatment (none inoculation), single inoculation with *Rhizobium* R14 and double inoculation R14+AMF4 and R14+ AMF2 significantly increased shoot dry weight and relative shoot dry weight up to 14, 13, and 17%, respectively. In addition, the comparison of control treatment, double inoculation with R14+AMF4 and single *Rhizobium* R14 inoculation were not significantly increased nitrogen uptake with 3.68 g N plant$^{-1}$ (Relative N-uptake 6% compared with control) and 3.61 g N plant$^{-1}$ (Relative N-uptake 4% compared with control). Phosphorus uptake was increased 0.31 g P plant$^{-1}$ (Relative P-uptake 19% compared with control. However, single rhizobium inoculation did not increase P-uptake and K-uptake of cowpea's shoot. In addition, the control treatment and double inoculation with R14+AMF3 recorded significantly higher K-uptake (Table 1).

**Table 1.** The effect of *Rhizobium* R14 and arbuscular mycorrhiza fungi (AMF) on relative shoot dry weight (Relative SDW), the uptake of the shoot nitrogen, phosphorus and potassium, relative nitrogen, phosphorus and potassium in cowpea no.14

| Treatments | SDW (g plant$^{-1}$) | Relative SDW (%) | N-uptake (g N plant$^{-1}$) | Relative N-uptake (%) | P-uptake (g P plant$^{-1}$) | Relative P-uptake (%) | K-uptake (g P plant$^{-1}$) | Relative K-uptake (%) |
|---|---|---|---|---|---|---|---|---|
| Control | 79.15 $^{c}$ | 100 | 3.48 | 100 | 0.26 | 100 | 0.46 $^{bc}$ | 100 |
| R14 | 90.54 $^{ab}$ | 114 | 3.61 | 104 | 0.23 | 88 | 0.32 $^{d}$ | 70 |
| R14+AMF1 | 86.06 $^{abc}$ | 109 | 2.99 | 86 | 0.27 | 104 | 0.44 $^{c}$ | 96 |
| R14+AMF2 | 92.39 $^{a}$ | 117 | 3.45 | 99 | 0.27 | 104 | 0.44 $^{c}$ | 96 |
| R14+AMF3 | 81.45 $^{bc}$ | 103 | 2.57 | 74 | 0.24 | 92 | 0.54 $^{a}$ | 117 |
| R14+AMF4 | 89.69 $^{ab}$ | 113 | 3.68 | 106 | 0.31 | 119 | 0.51 $^{ab}$ | 110 |
| Mean | 86.55 | | 0.30 | | 0.26 | | 0.45 | |
| *F*-test | * | | ns | | ns | | * | |
| C.V. (%) | 6.65 | | 16.30 | | 27.04 | | 8.85 | |

ns: non-significant, * significant at p≤0.05, ** significant at p≤0.01

Means with column letter within the same column is not significant by LSD at p≤0.05

SDW is shoot dry weight; N-uptake is Nitrogen uptake; P-uptake is Phosphorus uptake; K-uptake is Potassium uptake

R14 is the best rhizobium selected from the nodule of cowpea no.14

AMF1 is arbuscular mycorrhiza fungi from Samoeng Rice Research Center (orange spore)

AMF2 is arbuscular mycorrhiza fungi from Samoeng Rice Research Center (transparent spore)

AMF3 is arbuscular mycorrhiza fungi from Samoeng Rice Research Center (dark orange spore)

AMF4 is arbuscular mycorrhiza fungi from Department of Agriculture (Mixed spore)

**Root dry weight**: All inoculated treatment increased root dry weight compared to control treatment (Table 2). The highest root dry weight was found in single inoculated with rhizobium R14, that could increase root dry weight up to 61% compared with control. Root dry weight were decreased with dual inoculating with rhizobium no.14 and arbuscular mycorrhiza fungi (AMF).

Nodule dry weight: All treatments did not increase nodule dry weight as compared to control treatment. Double inoculating trended to decreased nodule dry weight compared with control treatment (Figure 1A).

**Table 2** The effect of *Rhizobium* R14 and arbuscular mycorrhiza fungi (AMF) on root dry weight, nodule dry weight, relative ureide index (RUI), and mycorrhizal colonization, of cowpea No. 14

| Treatments | RDW (g plant$^{-1}$) | Relative RDW (%) | Nod DW (g plant$^{-1}$) | Relative Nod-DW (%) | RUI (%) | Mycorrhizal colonization (%) |
|---|---|---|---|---|---|---|
| Control | 37.43 $^{c}$ | 100 | 0.82 $^{a}$ | 100 | 47.85 | 90 |
| R14 | 60.22 $^{a}$ | 161 | 0.75 $^{a}$ | 92 | 85.50 | 88 |
| R14+AMF1 | 53.23 $^{b}$ | 142 | 0.55 $^{b}$ | 67 | 60.21 | 78 |
| R14+AMF2 | 50.18 $^{b}$ | 134 | 0.48 $^{b}$ | 59 | 63.30 | 80 |
| R14+AMF3 | 50.18 $^{b}$ | 134 | 0.40 $^{b}$ | 49 | 52.07 | 80 |
| R14+AMF4 | 52.80 $^{c}$ | 114 | 0.74 $^{a}$ | 90 | 64.60 | 95 |
| Mean | 49.01 | 130 | 0.62 | | 57.15 | |
| F-test | ** | | ** | | ns | |
| C.V. (%) | 11.23 | | 17.02 | | 19.61 | |

ns: non-significant, * significant at p≤0.05, ** significant at p≤0.01

Means with column letter within the same column is not significant by LSD at p≤0.05

RDW is Root Dry Weight; Nod DW is Nodule Dry Weight (g plant-dry-weight$^{-1}$);

RUI is Relative Ureide Index; P-fix is Phosphorus fixation

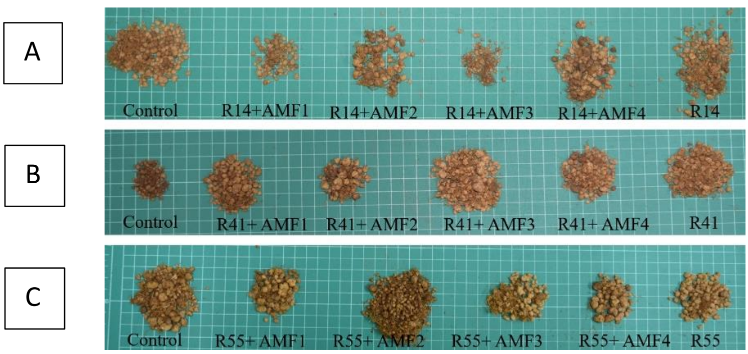


**Fig. 1** A nodule of cowpea no. 14 = A, no.41 = B, and no.55 = C

**Relative ureide index (%RUI):** The RUI was highest with rhizobium R14 inoculation. In addition, the double inoculating treatment trended to increased RUI (Table 2).

**Mycorrhizal colonization**: All treatments did not increase the percentage of mycorrhizal colonization compared to control, except double inoculating of AMF4 and rhizobium R14, which could increase the percentage of mycorrhizal colonization up to 95% (Table 2).

A positive correlation was found for correlation analysis on RUI vs. SDW and RUI vs. mycorrhizal colonization (Table 3).

**Table 3** Correlation between RUI and shoot dry weight (SDW) and between RUI and mycorrhizal colonization of cowpea no.14

| Correlation | N | r |
|---|---|---|
| RUI VS SDW | 6 | 0.908 * |
| RUI VS mycorrhizal colonization | 6 | 0.830 * |

RUI is Relative Ureide Index, SDW is shoot dry weight

In case of cowpea no.14, shoot growth can increase by only *Rhizobium* R14 applying. While, better N, P, K-uptake were showed by a co-inoculating between R14 and specific AMF groups. However, root dry weight and nodule dry weight were affective increased when only *Rhizobium* R14 using and have positive relationship with RUI. Moreover, RUI also have good effected to mycorrhizal colonization. These results showed the difference strain of rhizobium and AMF were specific to the plant host. Boddey et al. (2016) also stated that the cowpea could respond remarkably when highly effective rhizobium strain was used.

**2. Cowpea no. 41**

**Shoot dry weight, nitrogen, phosphorus and potassium uptake and their relative:** Compared with the control treatment (uninoculated), The comparison of all treatments significantly increased shoot dry weight and relative shoot dry weight 27-45%. In addition, compared with control treatment, double inoculation with R41+AMF4 was significantly increased nitrogen uptake with 4.04 g N plant$^{-1}$ (Relative N-uptake increased up to 52% compared with control) and also increased phosphorus uptake to 0.24 g P plant$^{-1}$ (Relative P-uptake increased to 41% compared with control treatment). Moreover, all inoculation treatment could increase the K-uptake of cowpea's shoot up to 23-34% compared with control and double inoculated R14+AMF4 (Table 4).

**Table 4.** The effect of *Rhizobium* R41 and arbuscular mycorrhiza fungi (AMF) on shoot dry weight, relative shoot dry weight (Relative SDW), nitrogen, phosphorus and potassium. The uptake of relative nitrogen, phosphorus and potassium uptake in cowpea no.41

| Treatments | SDW (g plant$^{-1}$) | Relative SDW (%) | N-uptake (g N plant$^{-1}$) | Relative N-uptake (%) | P-uptake (g P plant$^{-1}$) | Relative P-uptake (%) | K-uptake (g K plant$^{-1}$) | Relative K-uptake (%) |
|---|---|---|---|---|---|---|---|---|
| Control | 56.17 $^{c}$ | 100 | 2.67 $^{b}$ | 100 | 0.17 | 100 | 0.35 | 100 |
| R41 | 81.43 $^{a}$ | 145 | 3.04 $^{b}$ | 114 | 0.20 | 118 | 0.43 | 123 |
| R41+AMF1 | 71.40 $^{b}$ | 127 | 3.08 $^{ab}$ | 116 | 0.18 | 105 | 0.47 | 134 |
| R41+AMF2 | 78.60 $^{ab}$ | 139 | 3.18 $^{ab}$ | 119 | 0.19 | 112 | 0.47 | 134 |
| R41+AMF3 | 75.70 $^{ab}$ | 135 | 4.04 $^{a}$ | 152 | 0.24 | 141 | 0.44 | 126 |
| R41+AMF4 | 78.92 $^{ab}$ | 141 | 3.46 $^{ab}$ | 130 | 0.22 | 129 | 0.35 | 100 |
| Mean | 73.71 | | 3.44 | | 0.20 | | 0.42 | |
| F-test | ** | | ns | | ns | | ns | |
| C.V. (%) | 7.03 | | 18 | | 2.99 | | 19.66 | |

ns: non-significant, * significant at p≤0.05, ** significant at p≤0.01

Means with column letter within the same column is not significant by LSD at p≤0.05

SDW is shoot dry weight; N-uptake is Nitrogen uptake; P-uptake is Phosphorus uptake; K-uptake is Potassium uptake

R41 is the best rhizobium selected from nodule of cowpea no.41

**Root dry weight:** the single *Rhizobium* R41 inoculated treatment significantly increased root dry weight compared with control treatment up to 41.15% (Table 5), but other treatment did not affect the root dry weight cowpea no.41. Root dry weight was decreased whit dual inoculating with R14+ AMF.

**Nodule dry weight(g per plant dry weight):** All treatments did not increase dry nodule weight as compared to control treatment. Double inoculating trended to decreased nodule dry weight compared with control treatment (Figure 1B).

**Relative ureide index(%RUI):** The RUI of R14+AMF2 inoculation treatment was the highest. In addition, single rhizobium and double inoculating treatment trended to increased RUI compared with control.

Mycorrhizal colonization: All treatments increased the percentage of mycorrhizal colonization compared to control, except single rhizobium inoculating, which has decreased the percentage of mycorrhizal colonization less than control treatment (Table 5).

**Table 5** The effect of *Rhizobium* R14 and arbuscular mycorrhiza fungi (AMF) on root dry weight, nodule dry weight, relative ureide index (RUI), and mycorrhizal colonization of cowpea No. 41

| Treatments | RDW ($g\ plant^{-1}$) | Relative RDW (%) | Nod DW ($g\ plant^{-1}$) | Relative Nod-uptake (%) | RUI (%) | Mycorrhizal colonization (%) |
|---|---|---|---|---|---|---|
| Control | 40.63 $^{b}$ | 100.00 | 0.90 $^{a}$ | 100.00 | 43.95 | 90.00 |
| R41 | 57.35 $^{a}$ | 141.15 | 0.88 $^{a}$ | 97.78 | 54.19 | 87.50 |
| R41+AMF1 | 37.70 $^{bc}$ | 92.79 | 0.44 $^{b}$ | 48.89 | 53.06 | 95.00 |
| R41+AMF2 | 31.15 $^{cd}$ | 76.67 | 0.77 $^{b}$ | 30.00 | 61.81 | 90.00 |
| R41+AMF3 | 27.65 $^{b}$ | 68.05 | 0.80 $^{a}$ | 88.89 | 57.67 | 100.00 |
| R41+AMF4 | 34.81 $^{bcd}$ | 85.67 | 0.82 $^{a}$ | 91.11 | 54.56 | 97.50 |
| Mean | 38.21 | | 0.68 | | 51.11 | 1.32 |
| F-test | ** | | ** | | ns | ns |
| C.V. (%) | 15.06 | | 17.62 | | 19.26 | 19.41 |

ns: non-significant, * significant at p≤0.05, ** significant at p≤0.01

Means with column letter within the same column is not significant by LSD at p≤0.05

Nod DW is nodule dry weight

**3. Cowpea no. 55**

**Shoot dry weight, nitrogen, phosphorus and potassium uptake and their relative:** The comparison of the control treatment (uninoculated, R55+AMF1 and R55+AMF3 treatment increased shoot dry weight and relative shoot dry weight 1.18 and 5.57%. In addition, N-uptake and K-uptake of the R55+AMF3 inoculation were significantly increased to 40% and 41.03% compared to the control treatment. However, all treatments did not affect to increased P-uptake when compared with control (Table 7).

**Table 7** The effect of *Rhizobium* R55 and arbuscular mycorrhiza fungi (AMF) on shoot dry weight, relative shoot dry weight (Relative SDW), nitrogen, phosphorus and potassium uptake, relative nitrogen, phosphorus and potassium uptake of cowpea no.55

| Treatments | SDW (g plant$^{-1}$) | Relative SDW (%) | N-uptake (g N plant$^{-1}$) | Relative N-uptake (%) | P-uptake (g P plant$^{-1}$) | Relative P-uptake (%) | K-uptake (g K plant$^{-1}$) | Relative K-uptake (%) |
|---|---|---|---|---|---|---|---|---|
| Control | 83.36 $^{ab}$ | 100.00 | 3.25 $^{bc}$ | 100.00 | 0.25 | 100.00 | 0.39 $^{b}$ | 100.00 |
| R55 | 68.99 $^{c}$ | 82.42 | 2.58 $^{c}$ | 79.38 | 0.22 | 88.00 | 0.49 $^{ab}$ | 125.64 |
| R55+AMF1 | 84.69 $^{ab}$ | 101.18 | 3.70 $^{b}$ | 113.85 | 0.19 | 76.00 | 0.52 $^{a}$ | 133.33 |
| R55+AMF2 | 73.16 $^{c}$ | 87.41 | 2.82 $^{c}$ | 86.77 | 0.21 | 84.00 | 0.46 $^{ab}$ | 117.95 |
| R55+AMF3 | 88.36 $^{a}$ | 105.57 | 4.55 $^{a}$ | 140.00 | 0.25 | 100.00 | 0.55 $^{a}$ | 141.03 |
| R55+AMF4 | 75.89 $^{bc}$ | 90.17 | 2.83 $^{c}$ | 87.08 | 0.24 | 108.00 | 0.35 $^{b}$ | 89.76 |
| Mean | 79.13 | | 3.29 | | 0.26 | | 0.47 | |
| F-test | ** | | ** | | ns | | * | |
| C.V. (%) | 8.13 | | 15.60 | | 20.32 | | 16.66 | |

ns: non-significant, * significant at p≤0.05, ** significant at p≤0.01

Means with column letter within the same column is not significant by LSD at p≤0.05

R55 is the best rhizobium selected from the nodule of cowpea no.55

Root dry weight, nodule dry weight (Figure 1C), Relative ureide index (%RUI), and percentage of mycorrhizal colonization: compared with control, all inoculated treatments did not affect on root dry weight, nodule dry weight, relative ureide index (%RUI), and percentage of the root of cowpea no.55. Nevertheless, these parameters decreased by single rhizobium treatment and dual inoculation R14+AMF (Table 8).

**Table 8** The effect of *Rhizobium* R55 and arbuscular mycorrhiza fungi (AMF) on root dry weight, relative root dry weight (relative RDW), nodule dry weight (Nod DW), relative ureide index (RUI%) and mycorrhizal colonization of cowpea no. 55

| Treatments | RDW ($g\ plant^{-1}$) | Relative RDW (%) | Nod DW ($g\ plant^{-1}$) | Relative Nod-uptake (%) | RUI (%) | Mycorrhizal colonization(%) |
|---|---|---|---|---|---|---|
| Control | 74.51 [a] | 100.00 | 1.01 [a] | 100.00 | 48.39 | 95.00 |
| R55 | 26.72 [d] | 35.86 | 0.87 [a] | 86.14 | 55.03 | 90.00 |
| R55+AMF1 | 60.10 [b] | 80.66 | 0.42 [c] | 41.58 | 46.90 | 92.50 |
| R55+AMF2 | 54.70 [b] | 73.41 | 0.85 [a] | 84.16 | 55.03 | 100.00 |
| R55+AMF3 | 38.70 [c] | 51.94 | 0.67 [b] | 66.34 | 61.39 | 92.50 |
| R55+AMF4 | 38.41 [c] | 56.63 | 0.33 [c] | 32.67 | 49.48 | 92.50 |
| Mean | 48.87 | | 0.69 | | 51.11 | 1.33 |
| F-test | ** | | ** | | ns | ns |
| C.V. (%) | 11.58 | | 15.44 | | 19.26 | 20.99 |

ns: non-significant, * significant at $p \le 0.05$, ** significant at $p \le 0.01$

Means with column letter within the same column is not significant by LSD at $p \le 0.05$

Nod DW is nodule dry weight

**Number of branches per plant**

The result revealed that the amount of cowpea branch (no. 14 and no.41) were the highest by rhizobium inoculation treatment (5.5 and 5.75 branches, respectively). However, maximum of branch per plant of cowpea no. R55 was showed by using of combination R55+AMF4.

Control treatment of cowpea no.55 showed the highest nodule dry weight compared with rhizobium inoculation. This result might be due to rhizobium, which is non-host-specific bacteria that occurred in environmental areas (from water and air). There were more effected on nodule than rhizobium that inoculated to cowpea and in soil 2 times sterile (60 min per each time) by the mushroom sterilizer. Besides, rhizobium was non-spore microbe, so the soilborn natural rhizobium was eliminated by this procedure. In contrast, Rebika & Nongmaithem (2019) reported that inoculation of cowpea seed with rhizobium was found to be more nodule product when compared with no inoculation. Besides, the results found that the root dry weight of cowpea no.14 increased to 61% by comparing with the control. This might be due to *Rhizobium* R14 may can produce IAA that effect to improve cowpea root which is in an according with Lebrazi et al. (2020) reported that the IAA can produce by a majority of rhizobacteria. In addition, *Bradyrhizobium* sp. in root of cowpea that from South Africa and Mozambique also can produce IAA (Dabo et al., 2019). However, double inoculation rhizobium R14 and AMF did not improve the effectiveness of nitrogen fixation, shoot dry weight, %RUI, root dry weight, and nodule dry weight (except *Rhizobium* R14+AMF4 treatment showed no difference of dry nodule weight compared to rhizobium treatment). These results may be due to competition between *Rhizobium* R14 and AMF1-3.

%RUI is the nitrogen-fixing efficiency index of rhizobium. The results showed that %RUI of cowpea no.14, 41 and 55 in the control treatment have 47.85, 54.19 and 55.03%, respectively. In comparison, rhizobium inoculation treatment has a trend non-significant lower dry weight nodule than control. However, they showed better %RUI (85.50, 54.19 and 55.03%, respectively). Consequences, it referred to superior nitrogen-fixing efficiency from rhizobium, which has been selected from laboratory and they could more fixing nitrogen than control.

In this study found that mycorrhiza infection up to 90% in the control treatment. The results were affected by mycorrhiza fungi in the environment or the sterilization method of soil by mushroom sterilizer did not eliminate all microorganisms include natural arbuscular mycorrhiza.

## Conclusions

The effect of rhizobium and arbuscular mycorrhiza fungi (AMF) on cowpea's growth can conclude that all variety of cowpea was used in this research were good significantly respond to three rhizobium isolates. The only specific rhizobium used was affected by three cowpea varieties than combination application. The growth of cowpea, some nutrient uptake (such as nitrogen) which rhizobium inoculating had increased more than control. However, not all specific rhizobium and mycorrhizas can stimulate and enhance the growth of cowpea. Some co-inoculated may reduce their growh. Therefore, the good growth stimulation of cowpea that depends on the proper and specific selection of rhizobium and/or mycorrhizal fungi.

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