Application of Mycorrhiza-based biofertilizer to increase yields of several varieties of small chili intercropped with peanut or shallot

W Wangiyana, I K D Jaya and H Suheri

Post Graduate Program of the University of Mataram, Lombok, Indonesia

E-mail: w.wangiyana@unram.ac.id

Abstract. Mycorrhiza application in the nursery was reported to increase yield of “Dewata F1” chili in the field. This study aimed to examine the effect of mycorrhiza application in the nursery and intercropping with peanut or shallot in the field on growth and yield of several varieties of small chili. The experiment was arranged according to Split Split-Plot Design with three treatment factors, namely chili varieties as the main plots (V1= “Siung”, V2= “Sret”, V3= “Dewata F1”, V4= “Pelita F1”), mycorrhiza as the subplots (M0= without; M1= with mycorrhiza application), and intercropping as the sub-subplots (T0= monocrop, T1= intercropping with peanut, T2= intercropping with shallot). Results indicated that variety differences significantly affected all observation variables; so, did intercropping except for individual fruit weight, while mycorrhiza application significantly increased chili plant height, leaf number, fruit number, and fruit yield per plant. However, there were three-way interactions on fruit number and yield, with the highest fruit yield (55.96 g plant⁻¹) was in “Dewata F1” chili biofertilized with mycorrhiza and intercropped with shallot, and the lowest average (7.72 g plant⁻¹) was in monocropped “Sret” chili without mycorrhiza application. Therefore, nursery application of mycorrhiza is very important for high yield of chili in the field.

1. Introduction

Small chili (Capsicum frutescens L.) is a type of fruit vegetables whose fruit is directly consumed every day or as an ingredient in spices and chili sauce. Some of the chili varieties produce white and some green fruits when the fruits are still young, but when they are ripe the fruit color turns red. Besides its good taste, chili also contains lots of nutrients, in which every 100 g of red chili contains 32 Cal energy, 0.5 g protein, 0.3 g fat, 7.8 g carbohydrates, 1.6 g fiber, 29 mg Ca, 45 mg P, 0.5 mg Fe, 470 IU vitamin A, 0.05 mg thiamin, 0.06 mg riboflavin, 0.9 mg niacin, and 18 mg ascorbic acid [1].

Chili plants generally prefer dry climates to rainy seasons, and are more heat tolerant than tomatoes and eggplant. In the rainy season, chili plants are often attacked by several pathogens causing diseases, especially on the fruits. In addition, chili plants often fail to set fruits if they are exposed to continuous rainfall or when during flowering, the plants are exposed to heavy rains so that fruit production is generally very low in the rainy season because the flowers and small fruits will fall down, resulting in normally very low fruit production. This often causes the price of chili to be very high in the rainy season [1]. Therefore, the development of relevant technologies for chili production to make this crop more tolerant to environmental stress and yield more is very important.
Chili is one of the agricultural products needed every day by the people in Indonesia. With a high population reaching more than 300 million persons, it is not surprising that the needs for chili is very high. If the production is lower than the domestic demand, it will certainly cause an increase in the chili price, as often happened in the last several years. The price of chili reached Rp 160,000 per kg being higher than the price of beef in February 2017, as a result of the production failure in many locations, whereas the normal price was only around IDR 50,000 per kg [2].

In addition, red chili pepper has been proven to increase domestic inflation during 2016, with the largest contribution to inflation compared to other commodities [3], which indicates the importance in maintaining domestic production and supply of chili. To stabilize the chili supply meaning stabilization in production will be achieved by keeping its productivity always high. Therefore, it is highly necessary to create a productive and sustainable chili production technology. However, because they are also perishable agricultural products, chili fruits need post-harvest handling and processing technologies to extend the shelf life of the fruits. Thus, innovative research is needed in order to develop various technologies related to the production and supply of chili in a sustainable manner and giving the very important role of chili in the Indonesian economy so that chili will not contribute to high domestic inflation.

One of the reliable production technologies is utilization of plant symbiosis with arbuscular mycorrhizal fungi (AMF). The symbiosis with AMF causes plants to be more productive even under unfavorable environmental conditions such as water stress and low nutrient availability in the soil [4]. In relation to chili plants, the results of the research by Abror and Mauludin [5] showed that AMF inoculation significantly increased the number and weight of chili fruits and P uptake when compared to without AMF inoculation treatment. Research results from Dai et al. [6] also showed that inoculation of several AMF species and the addition of organic fertilizers significantly increased the dry weight of chili plants, and the addition of organic fertilizers further increased the dry weight of chili compared to control plants, whereas Sajan et al. [7] reported that reducing the NP fertilizer dose to 75% significantly decreased fruit yield of chili but when this treatment was accompanied by the application of Azospirillum sp (biological N₂ fixer) and AMF, it resulted in a highly significant increase in the dry fruit yield.

Nihayah et al. [8] also reported that the application of mycorrhiza-based biofertilizer significantly increased growth, fruit number and yields (fruit weight per plant) of “Dewata F1” chili variety, but when planting the chili plants with peanuts of “Sumbawa” local variety decreased yield of the chili fruit, when compared to monocrop chili in pot culture. However, several other studies have shown that legume crops, such as peanuts and soybeans, can increase growth rates and yield of non-legume crops in intercropping systems, as those reported by Inal et al. [9] on intercropping of peanuts with maize; Wangiyana et al. [10] on the intercropping of peanuts with shallots; Dulur et al. [11] on intercropping of peanuts with red rice in an aerobic irrigation system; and Wangiyana et al. [12,13] on additive intercropping of red rice with soybean in an aerobic irrigation system.

This study aimed to determine the effect of relay-planting (additive intercropping) of two peanuts or shallots rows between chili rows on growth and yield of several varieties of small chili transplanted following paddy rice crop on an irrigated rice field.

2. Materials and methods

This study used an experimental method and a field experiment was carried out from August to December 2017 in the experimental farm of the Faculty of Agriculture, University of Mataram, located Nyurlembang village, Narmada district of West Lombok, Indonesia.

2.1. Design of the experiment

The experiment was arranged according to the Split Split-Plot design by testing 3 treatment factors, namely: small chili varieties (V as the main plot factor) consisting V1= “Siung”, V2= “Sret”, V3= “Dewata F1”, and V4= “Pelita F1”; application of mycorrhiza biofertilizer in the nursery (M as the sub-plot factor) consisting of M0= without mycorrhiza and M1= with mycorrhiza biofertilizer; and
chili cultivation technique or additive intercropping (T as the sub sub-plot factor) consisting of T0= monocrop chili or without intercropping, T1 = intercropping chili-peanut by relay-planting two peanut rows between chili, T2 = relay-planting two rows of shallot between chili rows. Each treatment combination was replicated three times.

2.2 Implementation of the experiment
Before transplanting the chili seedlings onto the raised-beds, the pre-germinated seeds were first seeded in a seedling tray that has been filled with planting media in the form of a mixture (2:1) of soil and bokashi (EM4 fermented cattle manure) fertilizer that has been sterilized with autoclave. For the mycorrhizal seedlings (M1 treatment), application of mycorrhiza biofertilizer was done in this nursery by applying 5 grams of “Technofert” biofertilizer (a biofertilizer containing arbuscular mycorrhizal fungi) in the base (below the growing media) of the seedling holes in the tray, and the pre-germinated seeds were placed on the growing media and covered with a thin layer of growing media. After the seedlings were 2 weeks old, the young seedlings were transferred to (15 cm x 15 cm) poly-bags previously filled with the nursery media, which were then transplanted to the experimental beds after 5 weeks old. For the preparation of the beds, after harvesting the paddy rice in the Narmada experimental farm, soil tillage was done by ploughing and once harrowing using a hand tractor, then raised-beds of 3 x 1.8 m size were made for planting 5 rows of chili seedlings with a plant spacing of 60 x 45 cm. Between the raised-beds, furrows of 30 cm wide and 20-25 cm deep and 50 cm wide x 20-25 cm deep were made around and between blocks.

In the intercropping with shallots system (T2), the shallot planting with 15 cm in row spacing was carried out on the same day of transplanting chili, while peanut relay-planting with 22.5 cm in row spacing was done one week after transplanting chili seedlings. A total of two rows of peanuts or shallots were additively relay-planting between two rows of chili plants. Because this study aimed to examine the effect of relay-planting peanut plants on growth of chili, only 450 kg ha⁻¹ Phonska fertilizer (NPK 15-15-15) applied by dibbling 7 cm deep the fertilizer on 7 cm away from the planting holes at the 7 days after transplanting (DAT) chili seedlings. The only Phonska fertilizer was also applied for peanut and shallot on 7 days of age at the recommended doses of 200 kg ha⁻¹ for peanuts and shallots, respectively.

Weeding was done at the ages of 2, 4 and 7 weeks after planting (WAP) chili seedlings along with soil piling at 7 WAP for chili and peanut plants. Watering was done by flowing water through the furrows surrounding the raised-beds depending on the level of soil dryness. Pest control was done by alternately spraying Decis 25 EC and mixture of Amcothene 75 SP + Yasithrin 30 insecticides due to whitefly attacks. Harvesting chili was done only for the fruits being already red in color. The samples of 4 plants or clumps per plot were measured for yield component.

2.3. Observation variables and data analysis
The variables observed included growth and yield components of chili, which included plant height, number per plant of green leaves and branches, and fruits, fresh weight of fruits, and average weight per individual fruit. Data were analyzed with analysis of variance (ANOVA) and Tukey’s HSD at 5% level of significance, using the CoStat for Windows ver. 6.303. Analysis on correlation between variables was also carried out, i.e. using the Minitab for Windows Rel. 13.20.

3. Results and discussion
For a preliminary study on the compatibility of the “Technofert” biofertilizer containing mixed species of AMF, the pre-germinated seeds of Dewata F1 variety were grown for three weeks in a nursery container before transferred to growing media in polybags and transplanted five weeks after seeding, to be used in an experiment to examine intercropping treatments with various peanuts varieties. Before transplanting to the pots, AMF colonization of the inoculated chili root was found in the range between 70 to 75% at 5 weeks after seeding.
For the growth and yield of chili in the field (the summary results of the analysis of variance ANOVA presented in Table 1), indicated that among the three treatment factors, the difference in the chili variety was the most dominant factor affecting differences in growth and yield components of the chili plants, followed by cultivation techniques and application of mycorrhiza biofertilizer (AMF). Among the varieties tested, there were two groups of chili varieties, namely F1 hybrid (Dewata F1 and Pelita F1), and the non-hybrid superior varieties (Siung and Sret varieties). Among the three treatment factors, a significant three-way interaction occurred only on the number of fruits per plant and the total yield weight of chili fruits per plant (Table 1).

Table 1. Summary of ANOVA results for all growth and yield variables of small chili.

| Treatment factors | Plant height | Branch number per plant | Leaf number per plant | Fruit number per plant | Fresh fruit yield (g/plant) | Average individual fruit weight (g/fruit) | Coefficient of variation (%) |
|-------------------|--------------|-------------------------|-----------------------|------------------------|-----------------------------|-----------------------------------------|----------------------------|
| Varieties (Var)   | *            | ***                     | ***                   | ***                    | ***                         | ***                                     | 11.07                      |
| Mycorrhiza (Myc)  | ***         | ns                      | ***                   | ***                    | ***                         | ***                                     | 19.01                      |
| Myc * Var         | ns           | *                       | ns                    | ***                    | ns                          | ***                                     | 18.57                      |
| Intercropping (Int) | ***       | *                       | ***                   | ***                    | ns                          | ***                                     | 20.26                      |
| Int * Var         | ns           | ns                      | ***                   | *                      | ns                          | ***                                     | 24.26                      |
| Int * Myc         | ns           | ns                      | **                    | **                     | *                           | ns                                      | 19.64                      |
| Int * Myc * Var   | ns           | ns                      | ns                    | ***                    | **                          | ns                                      | ns                         |

Note: ns = non-significant (p-value >= 0.05); *, **, *** = significant at p-value <0.05; p-value < 0.01; p-value < 0.001, respectively.

Based on the three-way interaction, which is graphically shown in Figure 1 and 2, it can be seen that the responses vary widely between varieties in relation to the influence of other treatment factors. For example, chili plants inoculated with AMF (mycorrhiza-based biofertilizer) in the nursery consistently showed a higher number of fruits in the field, compared to those without AMF application except for the Siung variety, where the difference was less consistent (Figure 1).
In terms of the total fresh weight of fruit yield per plant, the three-way interaction (Figure 2), shows that chili plants inoculated with AMF in the nursery consistently showed higher yields of chili fruits compared to those not inoculated with AMF. This is different from the number of fruit because in the “Siung” variety, the average number of fruits in the T2 cultivation technique (intercropping chili with shallots) tended to be higher in chili plants without inoculated with AMF compared to those with AMF inoculations in the nursery (Figure 1).

**Figure 2.** Average fresh fruit yield of chili (g plant\(^{-1}\)) (Mean ± SE) as influenced by the three-way interaction among chili varieties (V1, V2, V3, V4), mycorrhiza (M0 = without, M1 = with mycorrhiza), and cultivation techniques (T0 = monocropped chili, T1 = intercropping with peanut, T2 = intercropping with shallot).

The higher yield or weight of chili fruits per plant in chili plants inoculated with AMF compared to those without AMF inoculation in the nursery was most probably due to the contribution of AMF that had developed in the roots of chili plants since in nursery. The difference in growth has indeed been seen since the chili seedlings still in the polybags, that the chili seedlings for all varieties inoculated with AMF appeared to be higher and with more leaves than those without AMF inoculations. This condition was carried over to the planting beds, that all chili varieties showed the taller plants for the seedlings inoculated with AMF compared to those without AMF inoculations in the nursery, as can be seen from Table 2, where the average plant height and number of leaves per plant are consistently higher in chili plants M1 compared to M0 treatments in the nursery.

The higher growth of AMF inoculated chili plants from seedling to the rest of the chilling growing period on the raised beds was most probably due to the positive effects of AMF on nutrient uptake by host plants, because the AMF hyphae were capable of helping their host plants take up more nutrients from the soil even when the roots could not reach the nutrients [4], which in turn resulted in higher number of leaves and plant height of the M1 than M0 chili plants. Results by Dai et al. [6] as well as by Sajan et al. [7] also clearly showed higher nutrient uptake by AMF-inoculated chili plants compared to the non-inoculated ones.

Based on the correlation analysis between the observation variables (Table 3), it can be seen that the chili fruit yield per plant (g plant\(^{-1}\)) has a positively significant correlation with plant height, and number of branches, leaves, as well as the number of fruits per plant. In addition, among the three growth variables of the chili plants, the number of leaves per plant contributed the highest to the fruit yield per plant with a determinant coefficient (R\(^2\)) of 37.95%. In relation to the influence of the main effect of AMF inoculation in the nursery, it can also be seen from Table 2 that plant height, number of branches, and leaf number per plant were significantly higher in chili plants inoculated with AMF in the nursery indicating a positive effect on the growth, as well as their yield components (total number and fresh weight of fruits per plant).
Table 2. Average branch number, leaf number, fruit number, and fresh weight of fruit per plant, plant height, and average weight per fruit of chili for each level of treatment factor.

| Treatments            | Plant height (cm) | Branch number per plant | Leaf number per plant | Fruit number per plant | Fruit yield per plant (g plant\(^{-1}\)) | Average fruit weight (g fruit\(^{-1}\)) |
|-----------------------|-------------------|-------------------------|-----------------------|------------------------|------------------------------------------|----------------------------------------|
| T0: Chili monocrop    | 52.5 b            | 4.1 b                   | 59.3 b                | 30.4 b                 | 23.03 b                                  | 0.80 a 1)                               |
| T1: Chili + peanut    | 62.1 a            | 4.2 ab                  | 88.5 a                | 32.1 b                 | 25.78 b                                  | 0.85 a                                 |
| T2: Chili + shallot   | 60.6 a            | 4.7 a                   | 79.2 a                | 42.0 a                 | 30.93 a                                  | 0.82 a                                 |
| HSD 0.05              | 4.6               | 0.6                     | 10.0                  | 5.0                    | 4.57                                      | 0.11                                   |
| M0: without myc       | 51.8 b            | 4.3 a                   | 61.2 b                | 24.7 b                 | 17.33 b                                  | 0.81 a                                 |
| M1: with mycorrhiza   | 65.0 a            | 4.4 a                   | 90.1 a                | 45.0 a                 | 35.83 a                                  | 0.83 a                                 |
| HSD 0.05              | 3.7               | 0.4                     | 5.6                   | 3.0                    | 3.01                                      | 0.07                                   |
| V1: Siung             | 65.7 a            | 4.4 a                   | 104.6 a               | 55.5 a                 | 37.03 a                                  | 0.68 c                                 |
| V2: Sret              | 57.6 ab           | 3.4 b                   | 71.0 b                | 20.6 c                 | 17.57 c                                  | 0.87 b                                 |
| V3: Dewata F1         | 49.8 b            | 4.9 a                   | 71.0 b                | 41.1 b                 | 29.47 b                                  | 0.74 c                                 |
| V4: Pelita F1         | 60.5 ab           | 4.7 a                   | 56.1 c                | 22.3 c                 | 22.26 c                                  | 1.01 a                                 |
| HSD 0.05              | 13.0              | 0.6                     | 10.3                  | 8.4                    | 4.79                                      | 0.11                                   |

1) Mean values in each column followed by the same letter indicate non-significant differences between levels of a treatment factor.

From the main effect, the positive effect of AMF inoculation in the nursery on the chili fruit yield per plant in the field can be seen from Table 2, in which the average fruit yield per plant is significantly higher (even more than twice) compared to those without AMF inoculation. Other similar results have also been reported by Abror and Mauludin [5] that AMF inoculation on chili significantly increased the number and weight of chili fruits and P uptake compared to without AMF inoculation treatment. In addition, Agustin [14] also reported that AMF isolates from ex- chili planting field (after being inoculated on young plants of big chili) were able to significantly increase the number and weight of fruits and the uptakes of N, P, and K by the inoculated plants. Furthermore Wangiyana et al. [15] also found that the application of AMF on some promising lines of red rice not only increased grain yields, but also the anthocyanin contents in the husked grains of the plants.

Table 3. Coefficients of correlation between observation variables, each with its p-value.

| Observation Variables | Plant height | Branch number per plant | Leaf number per plant | Fruit number per plant | Fresh fruit yield (g plant\(^{-1}\)) |
|-----------------------|--------------|-------------------------|-----------------------|------------------------|--------------------------------------|
| Branch number per plant | 0.160        | 0.179                   |                       |                        |                                      |
| p-value               | 0.514        | 0.380                   |                       |                        |                                      |
| p-value               | 0.386        | 0.225                   | 0.630                 |                        |                                      |
| p-value               | 0.001        | 0.057                   | 0.000                 | 0.000                  |                                      |
| Fresh fruit yield (g plant\(^{-1}\)) | 0.455       | 0.238                   | 0.616                 | 0.883                  |                                      |
| p-value               | 0.000        | 0.044                   | 0.000                 | 0.000                  |                                      |
| Average fruit weight  | -0.022       | -0.096                  | -0.323                | -0.510                 | -0.099                               |
| p-value               | 0.852        | 0.421                   | 0.006                 | 0.000                  | 0.409                                |

The positive effect of additive intercropping with peanuts or shallots on growth (plant height and number of leaves) and chili fruit yields per plant (Table 2), especially on the chili plants inoculated with AMF (Figure 1), was most probably associated with an increase in the N and P uptakes compared to monocropped chili plants. From the research results reported by Sajan et al. [7] that a reduction in
the NP fertilization dosage to 75% of significantly reduced chili fruit yield, but when this treatment was accompanied with the applications of N₂ fixer (Azospirillum sp) and AMF, these microbes very significantly increased fruit yield of the chili plants. AMF inoculation and intercropping with peanuts were also reported to significantly increase growth and yield of shallot plants [10], indicating that shallot plants are highly responsive to AMF symbiosis. Inal et al. [9] reported that the availability of various macro nutrients was much higher in the rhizosphere of intercropped maize and peanuts compared to their monocrops, leading to higher nutrient uptake in the intercropped plants. With radioactive techniques, some researchers have also proven that there is a significant transfer of N from legume to non-legume plants and the transfer rate is higher if symbiosis with AMF is involved, in which AMF hypha infects the roots of the two plants in the intercropping system, especially from legumes to cereal plants or non-legumes in crop mixtures [16, 17].

Although based on the main effect there were significant differences in the number of fruits and fresh weight of fruit yield per plant between the varieties tested (Table 2), there was also a significant interaction between the varieties and AMF inoculation, which means that there were differences in response to AMF inoculation between varieties. From Figure 3, it can be seen that the average number of chili fruits per plant in “Siung” variety was not significantly different between AMF and non-AMF inoculated chili plants but very significant differences in the other three varieties.

![Figure 3](image_url)

**Figure 3.** Average total number of fruits per plant (Mean ± SE) between treatments indicating a significant two-way interaction between chili varieties and mycorrhiza application in the nursery (M0= without, M1= with mycorrhiza).

4. **Conclusions**

It can be concluded that all treatment factors had significant effects on yield component of small chili with variety differences imposed the most dominant effects. However, there were three-way interaction effects on number of fruits and fruit yield per plant in which the highest fruit yield average (55.96 g plant⁻¹) was in “Dewata F1” chili inoculated with AMF in the nursery and intercropped with shallots in the field and the lowest average (7.72 g plant⁻¹) was on monocropped “Sret” chili without mycorrhiza application in the nursery. Therefore, application of mycorrhiza biofertilizer in the nursery is very important in increasing growth and yield of chili plants in the field, and application of mycorrhiza biofertilizer is much easier in the nursery than in the field.

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