Arbuscular mycorrhiza increased N, P, K, and Fe uptake, growth and yield of vegetables grown on Andisols with different rates of NPK fertilizers

A Mbusango¹, A Nurbaity¹, B N Fitriatin¹, M A Solihin¹, and N Istifadah¹

¹ Faculty of Agriculture, Universitas Padjadjaran
Jl. Raya Bandung Sumedang km. 21, Jatinangor, West Java, Indonesia

Email: aminpuma126@gmail.com; a.nurbaity@unpad.ac.id

Abstract. Arbuscular mycorrhizal fungi (AMF) is a biological fertilizer that can function as an ameliorant which capable in increasing plant growth and yield of plants. Some vegetables such as kangkong (Ipomoea aquatica) and lettuce (Lactuca sativa) grown in Indonesia using substantial amount of NPK lettuce which has an impact on land degradation. The aim of this experiment were to determine whether AMF application technology can reduce the use of NPK application and can increase nutrient uptake of N, P, K, Fe, growth and yield of kangkong and lettuce grown on Andisols. This experiment was carried out at Screen House in Lembang District, West Java. This study used an experimental method with split plot design with two levels and three replications and applied on two types of vegetable plants. The main plot was AMF application (without and with AMF) and sub plot was various dosages of NPK (0, 25, 50, 75, 100%) of recommended dosages. Results of this study indicated that the application of AMF increased nutrient uptakes, growth and yield of vegetables. The AMF showed highest values at a dosages of 50% NPK, in terms of parameters of plant height, leaf number, N, P, K uptakes, chlorophyll contents, fresh root weights, root lengths and total mycorrhizal root lengths. Consequently, AMF was effective at low NPK dosages and was able to reduce the use of NPK dosages up to 50%. In conclusion, AMF has potential to be used in sustainable agriculture system

1. Introduction
Kangkong and lettuce are vegetable plants that have a high consumption rate. In the last five years, the production of kangkong and lettuce in Indonesia has been decreased [1]. Due to limited land resources caused by conversion of agricultural land to non-agricultural land and land degradation due to use of chemical fertilizer, there is a need to find alternative solution to solve the problem in horticulture production, including vegetables such as kangkong and lettuce.

The occurrence of agricultural land for horticulture production such as Andisols is largely available [2]. Andisols has important characteristics due to its high capacity to immobilize phosphorus, and high use of NPK fertilizer to increase soil fertility to achieve maximum yield and production is common [3].

NPK fertilizer have been widely used by horticulture farmers. The use of NPK continuously will have an impact on land degradation, that inhibits plant growth, yield and biomass [4] and cause toxins to organisms that live in plant rhizosphere [5]. One of approaches to reduce the use of chemical fertilizer in vegetables cultivation is through the application of biofertilizer [6].
One important organism that acts as a biological fertilizer was the arbuscular mycorrhizal fungi AMF. Arbuscular MF plays vital role in expanding function of the root system in obtaining water [7] and nutrient absorption in the soil [8] thereby increasing plant growth and yield of plants [9]. Some works showed that application of AMF on plants reduced the use of NPK dosages up to 50% on potato plants [6] and increased nutrients uptake [10] including N and P [11]. The aims of this research was to determine whether the application of AMF could reduce the NPK fertiliser use and increase the growth and yield of vegetables, especially kangkong and lettuce.

2. Materials and Methods
This research was conducted at Screen house Lembang West Java, Indonesia (1312 m above sea level), and Laboratory of Soil Biology and Soil Fertility Laboratory, Department of Soil Sciences, Faculty of Agriculture, Universitas Padjadjaran. The properties of the soil are presented in Table 1.

Table 1. Analysis of soil properties.

| Properties      | Mean value |
|-----------------|------------|
| pH H2O          | 5.25       |
| Organic Carbon (%) | 3.55      |
| Total N (%)    | 0.5        |
| Available P (ppm) | 131.07    |
| Available K    | 4.15       |
| CEC (cmol.kg\(^{-1}\)) | 40.71     |
| Base Saturation (%) | 30.85     |
| Sand (%)       | 22         |
| Silt (%)       | 50         |
| Clay (%)       | 28         |
| Soil Texture   | clay loam  |

This study used an experimental method with split plot design with two levels and three replications, for each types of vegetable plants (kangkong and lettuce). The first treatment was AMF (without and with) and the second treatment was various dosages of NPK, ie. 0%, 25%, 50%, 75% and 100% of recommended rates (equal to 0, 75, 150, 225 and 300 kg ha\(^{-1}\)).

Arbuscular MF used were from the collection of Soil Biology Laboratory, Faculty of Agriculture, Universitas Padjadjaran (mixture of Glomus sp. and Gigaspora sp.). Application of AMF was conducted by giving 50 g of AMF inoculants with a density of 300 spores g\(^{-1}\), on planting holes, 3 days before planting. The NPK fertilizer was given according to the treatment arrangements, at the time of planting.

Kangkong seeds obtained from PT East West Seed Indonesia and lettuce from PT New Day Seed Indonesia. The seeds were grown in the media with the mixture of soils: rice husk: zeolite as described by [12]. Before sowing, the soil was sterilized in autoclave with pressure 1 atm and a temperature 121° C. Seedlings were then planted in field soil with AMF and NPK treatments in a screen house with natural light interception, and watered every day to maintain filled capacity. The size of each plot was 1 m x 1 m and space between plots was 50 cm. Total area of the field plot was 45 m x 5 m.

Observation parameters evaluated were: plant weight at 7 weeks after planting (WAP), mycorrhizal root colonization and N, P, K, and Fe uptake. Percentage of infection is calculated using the gridline intersection method. Nutrient concentrations were analyzed using plants in the vegetative phase 7 week after plant (WAP), including N content determined by Kjeldhal method, P by the dry ashing method, while for K and Fe using Atomic Absorption Spectrometer. All data collected were analyzed using two-way ANOVA with SPSS software (version 24). Differences at the 5% significance level were tested using Duncan's Multiple Range Test.
3. Results and Discussion

3.1. Mycorrhizal Parameters

An assessment indicator to see the effect of AMF on plants was by calculating the percentage of root colonization, root length, and root length colonized. The effect of AMF colonization and NPK fertilizers on kangkong and lettuce can be seen in Table 2 and Table 3.

Table 2. Root colonization of kangkong roots in combination treatment of AMF with various dosages of NPK.

| Status of AMF | Dosages of NPK (%) | Root colonization | Root colonization |
|---------------|--------------------|------------------|------------------|
|               |                    | Colonization %   | Root Length (cm) | % of Root Length Colonized (cm<sup>-1</sup>) |
| Mycorrhiza    | 0                  | 31.20 b          | 156.40 a         | 76.90 b          |
|               | 25                 | 31.50 b          | 269.50 d         | 139.00 c         |
|               | 50                 | 32.10 b          | 291.20 e         | 223.0 e          |
|               | 75                 | 29.50 a          | 211.90 c         | 166.30 d         |
|               | 100                | 28.60 a          | 191.10 b         | 73.40 a          |
| Non Mycorrhiza| 0                  | 27.70 ab         | 8.50 a           | 3.10 a           |
|               | 25                 | 27.20 a          | 12.60 ab         | 4.80 ab          |
|               | 50                 | 28.10 ab         | 16.20 b          | 7.00 c           |
|               | 75                 | 28.60 ab         | 14.90 b          | 5.60 bc          |
|               | 100                | 28.90 b          | 11.67 ab         | 5.70 bc          |
| Main Effects  | AMF                | <0.001           | <0.001           | <0.001           |
|               | NPK                | <ns>              | <0.001           | <0.001           |
| Interaction Effect | AMF X NPK | <0.001          | <0.001           | <0.001           |

Within each column, means followed by different letter are significantly different (p< 0.001), ns: not significant

Table 2 showed the highest results in the combination of AMF and 50% NPK dosages against the percentage parameters of root colonization, root length and the percentage of colonized root length. In general, the overall parameters of root colonization in kangkong plants have had positive interactions between AMF x NPK (<0.001).

Table 3. Colonization of lettuce plant roots in combination treatment of AMF with various dosages NPK.

| Status AMF | Dosages of NPK (%) | Root colonization (7 WAP) | Root colonization |
|------------|--------------------|---------------------------|------------------|
|            |                    | Colonization %     | Root Length (m)  | % of Root Length Colonized (m<sup>-1</sup>) |
| Mycorrhiza | 0                  | 28.00 ab           | 27.10 a          | 9.30 a           |
|            | 25                 | 29.40 bc           | 25.60 b          | 10.80 b          |
|            | 50                 | 30.20 c            | 43.30 c          | 13.60 c          |
|            | 75                 | 27.80 ab           | 37.40 b          | 11.10 b          |
|            | 100                | 26.60 a            | 26.40 ab         | 10.20 ab         |
| Non Mycorrhiza | 0                  | 22.90 a            | 6.40 ab          | 5.30 ab          |
|            | 25                 | 24.60 a            | 7.70 b           | 5.50 b           |
|            | 50                 | 24.30 a            | 9.60 ab          | 4.40 ab          |
|            | 75                 | 24.20 a            | 10.70 a          | 4.10 a           |
|            | 100                | 23.60 a            | 8.70 ab          | 5.10 ab          |
| Main Effects | AMF                | <0.05             | <0.001           | <0.001           |
|            | NPK                | <0.001            | <0.001           | <0.001           |
| Interaction Effects | AMF X NPK | <ns>              | <0.001           | <0.001           |

Within each column, means followed by different letter are significantly different (p< 0.05), ns: not significant
Based on statistical tests on the parameters of colonization of the roots of lettuce plants showed consistent results in the combination of AMF and 50% NPK in all parameters, namely the percentage of root colonization, root length and percentage of colonized root length on Table 3. The parameters of percentage colonization root did not occur between AMF x NPK (<ns) but showed a positive interaction between AMF x NPK (<0.001) on parameters of root length and the percentage of colonized root length.

### 3.2. N, P, K, and Fe Uptake of Plants

Based on the results of the research in Table 4, it showed that the interaction of the combination treatment of AMF with a dosages of 50% NPK gave the best results on the uptake of N, P, and K in kangkong plants. The highest uptake of Fe was shown in NPK treatment without AMF at 100%. This can be seen from the average high nutrient uptake of kangkong in the parameters observed such as: N uptake (0.08%), P (0.07%), K (5.12%) in the treatment using AMF, and Fe (0.63%) in treatment not using AMF. There was a positive interaction between AMF x NPK (<0.001) on the parameters of N, P, K, and interactivity (<0.05) between AMF x NPK and the parameters of Fe absorption.

#### Table 4. NPK and Fe uptake of kangkong plant as the effect of AMF and various dosages of NPK.

| Status AMF   | Dosages of NPK (%) | Nutrient Uptake |
|--------------|--------------------|-----------------|
|              |                    | N (%) | P (%) | K (%) | Fe (%) |
| Mycorrhiza   | 0                  | 0.04 a | 0.03 a | 4.11 a | 0.50 a |
|              | 25                 | 0.05 b | 0.06 c | 4.32 b | 0.51 ab|
|              | 50                 | 0.08 e | 0.07 c | 6.52 d | 0.48 a |
|              | 75                 | 0.06 d | 0.05 b | 5.12 c | 0.52 ab|
|              | 100                | 0.06 c | 0.04 b | 5.02 c | 0.56 b |
|              | 0                  | 0.04 a | 0.03 a | 4.46 a | 0.51 a |
|              | 25                 | 0.05 b | 0.04 a | 4.40 a | 0.53 a |
| Non Mycorrhiza| 50                | 0.05 bc| 0.04 a | 4.89 b | 0.62 b |
|              | 75                 | 0.05 c | 0.04 a | 4.97 b | 0.60 b |
|              | 100                | 0.05 c | 0.04 a | 5.21 c | 0.63 b |
| Main Effects | AMF                | <0.001 | <0.05 | <0.05 | <0.05 |
|              | NPK                | <0.001 | <0.001 | <0.001 | <0.001 |
| Interaction Effects | AMF X NPK | <0.001 | <0.001 | <0.001 | <0.001 |

Within each column, means followed by different letter are significantly different ($p < 0.001$)

#### Table 5. NPK and Fe uptake of lettuce plant as the effect of AMF and various dosages of NPK.

| Status AMF   | Dosages of NPK (%) | Nutrient Uptake |
|--------------|--------------------|-----------------|
|              |                    | N (%) | P (%) | K (%) | Fe (%) |
| Mycorrhiza   | 0                  | 0.05 a | 0.19 b | 3.36 a | 0.83 a |
|              | 25                 | 0.05 a | 0.20 b | 3.59 bc| 0.88 ab|
|              | 50                 | 0.06 b | 0.27 c | 3.45 ab| 0.89 ab|
|              | 75                 | 0.05 a | 0.19 b | 3.48 ab| 0.93 b |
|              | 100                | 0.05 a | 0.16 a | 3.66 c | 0.91 b |
|              | 0                  | 0.04 a | 0.15 a | 3.23 a | 1.04 c |
|              | 25                 | 0.05 a | 0.16 a | 3.65 b | 1.04 c |
| Non Mycorrhiza| 50                | 0.05 a | 0.16 a | 3.74 bc| 0.97 b |
|              | 75                 | 0.05 a | 0.19 b | 3.78 bc| 0.82 a |
|              | 100                | 0.05 a | 0.20 b | 3.89 c | 0.86 a |
| Main Effects | AMF                | <0.05 | <0.001 | <ns    | <0.001 |
|              | NPK                | <0.001 | <0.001 | <0.001 | <0.001 |
| Interaction Effects | AMF X NPK | <0.001 | <0.001 | <0.001 | <0.001 |

Within each column, means followed by different letter are significantly different ($p < 0.05$)

Results of the test on lettuce plant in Table 5 showed the highest value was shown in combination treatment of AMF and 50% NPK. N uptake (0.057%), P (0.271%), and K uptake (3.884%) were highest.
at 100%, while Fe uptake (1.039%) at 0% dosages of NPK without AMF. However, it has positive interaction between AMF x NPK (<0.001) for uptake of N, P, K and Fe in lettuce plants.

3.3. Yield of Vegetables
There was a statistically significant interaction between AMF x NPK (p <0.001). Mycorrhizal treatment and 50% of NPK showed the highest values, namely fresh weight (53.3 g) at on root and (147.0 g) in canopy of kangkong (Table 6).

Results of study in Table 7 showed that giving AMF had the best effect on parameters of fresh weight (110.3 grams) and 50% NPK and 10.0 gram fresh weight parameters. The NPK at 50% dosages also did not different at a dosages of 75% NPK. Significant interactions between AMF x NPK (<0.001) in shoot parameter and root parameters interacted between AMF x NPK (<005) in lettuce plants.

| Status AMF | Dosages NPK (%) | Fresh Weight of Kangkong (gram) |
|------------|-----------------|---------------------------------|
|            |                 | Shoot                           | Root                           |
| Mycorrhiza | 0               | 89.8 a                          | 36.6 a                          |
|           | 25              | 106.6 b                         | 39.2 b                          |
|           | 50              | 147.0 c                         | 52.3 d                          |
|           | 75              | 131.2 d                         | 50.5 d                          |
|           | 100             | 122.5 c                         | 44.4 c                          |
|           | 0               | 87.7 a                          | 38.1 a                          |
|           | 25              | 89.8 a                          | 40.7 b                          |
| Non Mycorrhiza | 50         | 101.8 b                         | 41.5 b                          |
|           | 75              | 102.0 b                         | 44.9 c                          |
|           | 100             | 122.7 c                         | 48.1 d                          |
| Main Effects | AMF         | <ns                             | <0.001                          |
|            | NPK             | <0.001                          | <0.001                          |
| Interaction Effects | AMF X NPK | <0.001                          | <0.001                          |

Within each column, means followed by different letter are significantly different (p< 0.001). ns: Not significant

| Status AMF | Dosages of NPK (%) | Fresh Weight of Lettuce (gram) |
|------------|--------------------|--------------------------------|
|            |                    | Shoot                           | Root                           |
| Mycorrhiza | 0                  | 86.8 a                          | 8.3 a                          |
|           | 25                 | 91.0 a                          | 8.9 ab                         |
|           | 50                 | 110.3 b                         | 10.0 c                         |
|           | 75                 | 89.1 a                          | 9.5 bc                         |
|           | 100                | 84.6 a                          | 8.8 ab                         |
|           | 0                  | 61.8 a                          | 3.2 a                          |
|           | 25                 | 69.2 ab                         | 3.9 a                          |
| Non Mycorrhiza | 50         | 74.8 b                          | 4.1 a                          |
|           | 75                 | 83.7 c                          | 4.0 a                          |
|           | 100                | 89.01 c                         | 5.4 b                          |
| Main Effects | AMF        | <0.001                          | <0.001                          |
|            | NPK             | <0.001                          | <0.001                          |
| Interaction Effects | AMF X NPK | <0.001                          | <0.05                           |

Within each column, means followed by different letter are significantly different (p< 0.001)

From the results above, it showed that AMF isolates could become biological fertilizers that could be used to support optimal nutrient availability in acidic soils. This was supported by finding that role of mycorrhizal fungi for host plants is to enlarge the area of root hair uptake through a formation of mycelium around the roots [12]. Due to the expansion of root range area through the help of mycelium, AMF causes more nutrients that can be absorbed by the host plant compared to other plants that are not
symbiotic with AMF. Arbuscular MF can increase nitrogen and phosphorus uptake in drought conditions [7], increase plant biomass by more than 170% [11], and reduce on use of NPK to 50% [6]. Tables 6 and 7, have shown evidence that both kangkong and lettuce species using a combination of AMF and NPK were given a dose of 75% to 50% for kangkong even reaching a dose of 25% in lettuce plants against on parameters of plant wet weight.

In this experiment, root colonization parameter gave the best response at recommended combination level of NPK (150 kg ha\(^{-1}\)) with AMF to the yield of kangkong and lettuce. The results of kangkong and lettuce which have different leaf types show same significant value to the parameters of root colonization, uptake and base weight of the plant. pH Acidic soil (5.25), reflecting low fertility, causes AMF to be more symbiotic with host plants, thus providing the best response to increased yields and production, indicated by wet weight values. This finding in line with [13], who stated that AMF increased 30-fold in pH acidity (4.5).

Parameters on fresh weight of kangkong and lettuce plants which showed AMF was more effective in increasing yield and production compared to plants without using AMF. It is reflected that AMF can develop symbiosis of kangkong and lettuce plants in a combination treatment of AMF and 50% NPK. Parameters of 50% nutrient uptake (150 kg ha\(^{-1}\)) can increase N fixation, P uptake, K uptake and is able to suppress Fe uptake in acidic pH Andisols. Arbuscular MF can increase P and N nutrient uptake so it can spur plant growth and increase pH in soils that have very acidic pH [14].

4. Conclusion
Arbuscular fungi mycorrhiza application reduces NPK usage up to 50%, where root colonization, N, P, K uptake increases, while Fe uptake decreases. It also increased yields of kangkong and lettuce. Using a combination of FMA and 50% NPK has the same effect as the highest recommendation level of NPK used by local farmers.

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