Application of Vesicular Arbuscular Mycorrhiza to the Growth and Essential Oil Content of Rosemary (*Rosmarinus officinalis* L.)

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ABSTRACT

Vesicular arbuscular mycorrhiza (VAM) is one of the fungi groups that lives in soil. VAM plays a major role to increase nutrition and water absorption, and the production of plant growth substances. This research was conducted to determine the optimal dose of vesicular arbuscular mycorrhiza (VAM) on the growth and essential oil content of rosemary (Rosmarinus officinalis L.). The experimental design used in this study was a set of randomized complete block design (RCBD) with four replications. The factor was dose of VAM 0, 5, 10, 15, 20 g/plant. Data were analysed using of variance (ANOVA), variables indicating the significant different were analysed by orthogonal polynomial. The result showed the dose of VAM significantly affected stem diameter (mm) and affected highly significant on plant heigh (cm) and essential oil content of rosemary (%). Application VAM 20 g/plant was recommended to cultivate of rosemary.

Keywords: *essential oil, growth, rosemary, vesicular arbuscular mycorrhiza.*

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is one of the essential oils producing plant species. The essential oil content in rosemary plants is widely used in the world of health, besides that rosemary plants are often used as aromatherapy because there are essential oils that have a positive impact on the body. In the research of Hendawy et al., the essential oil content produced in rosemary plants are 1,24% and 1,41%. The application of vesicular arbuscular mycorrhiza can also affect essential oil content (1).

The application of VAM in the cultivation of some herbs has been shown to be able to significantly increase the essential oil contents of these (2,3,4). However, until now there is no studies have been conducted on the effect of the VAM on rosemary plants. The purpose of this study was to study the effect of the adding VAM to essential oil content produced by studying the application of VAM on the growth of rosemary plants.

MATERIAL AND METHODS

This research was conducted from December 2021 to March 2022. Location research is at Rumah Atsiri Indonesia Watusambang, Plumbon, Tawangmangu District, Karanganyar Regency, Central Java with an altitude of ± 860 m above sea level. Research for destilation of essential oil content was carried out from March to April 2022 at the Laboratory of the Faculty of Agriculture, Tidar University.

The tools used in this study were polybags, shovels or scoops, rulers, meters, calipers, digital scales, plastics, distillers, beaker glass, burettes, electric stoves, distillation flasks, thermometers,

measuring cups, and plant shears. While materials needed are rosemary seeds obtained from Rumah Atsiri Indonesia, vesicular arbuscular mycorrhiza from CV. Abadi Sejahtera Malang, water, soil, manure, husks, aquadest, and anhydrous Na_2SO_4.

The experimental design used in this study was a set of randomized complete block design (RCBD) with four replications. The factor was dose of VAM D0: 0 g/plant, D1: 5 g/plant, D2: 10 g/plant, D3: 15 g/plant, D4: 20 g/plant. Data were analysed using of variance (ANOVA), variables indicating the significant different were analysed by orthogonal polynomial.

The stages of this research are mycorrhiza preparation, cultivation preparation, rosemary seed preparation, cultivation, rosemary maintenance, and observe research parameters.

Observations were made on each treatment consisted of 4 samples. The parameters of the observations made in this study refer to Azizah research, which are increase in plant stem height (cm), increase in stem diameter (mm), increase in the number of branches, root length (cm), root amount, fresh weight of upper plant (g), root fresh weight (g), and essential oil content (%) (3). The essential oil content of rosemary was obtained by distillation on plant samples, in this research used steam method. After obtaining the volume of essential oils, the total oil content is calculated using the calculation formula according to [4] as follows:

$$\text{Total oil content (\%v/w)} = \frac{\text{volume of essential oi}}{\textit{weight of simplisia}} \text{x} 100\%$$

RESULT

The result showed that the VAM treatment had an effect on growth and essential oil content of rosemary. Based on the result of analysed using of variance, VAM had a very significant effect on the increase in plant stem height and essential oil content, and had a significant effect on the increase in stem diameter **(Table 1)**.

Table 1. F value calculates how VAM doses affect the growth and essential oil content of rosemary.

Research parameters	F count
Increase in plant stem height (cm)	**6,24****
Increase in the number of branches	0,31^{ns}
Increase in stem diameter (mm)	**3,02***
Root length (cm)	1,36^{ns}
Root amount	1,96^{ns}
Fresh weight of upper plant (g)	0,86^{ns}
Root fresh weight (g)	1,23^{ns}
Essential oil content (%)	**5,41****

Information
** : significantly affected highly
* : significantly affected
ns : insignificant

After knowing the results of F count, further test of orthogonal polynomials is carried out on significant parameters, that is increase in plant stem height (cm), increase in stem diameter (mm), and essential oil content (%). **(Figure 1)**, **(Figure 2)**, **(Figure 3)**

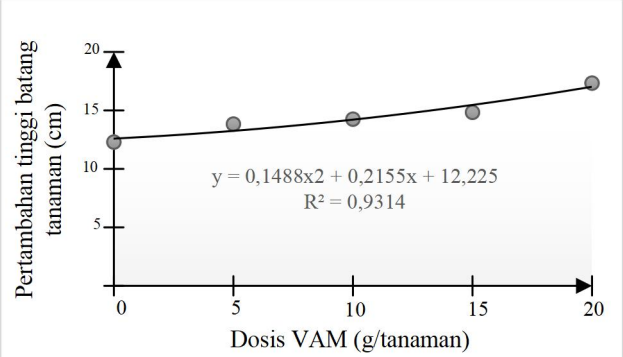

Figure 1. Effect of VAM dose on rosemary stem height increase

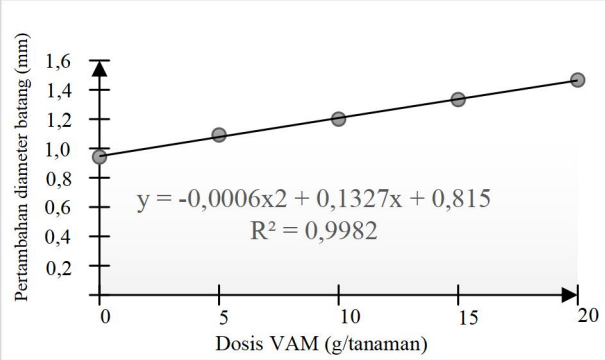

Figure 2. Effect of VAM dose on rosemary stem diameter increase

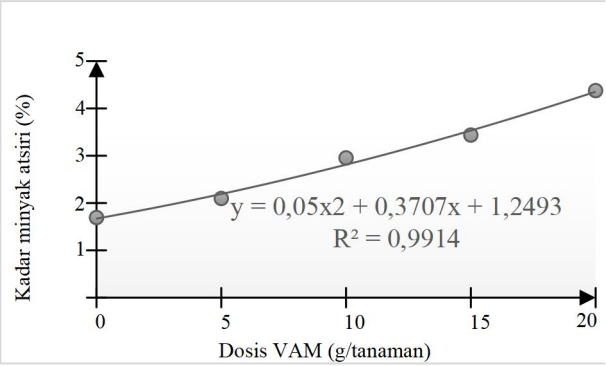

Figure 3. Effect of VAM dose on essential oil content of rosemary

DISCUSSION

1. Application of Vesicular Arbuscular Mycorrhiza (VAM) Affected Highly Significant on Rosemary Stem Height Increase

The application of VAM to rosemary affected the height of rosemary and it was found that the addition of VAM was able to increase the height of rosemary. VAM dose of 20 g/plant resulted in an increase in plant diameter of 17,33 cm which was the highest compared to other doses, so the application VAM 20 g/plant can increase the stem height of rosemary.

The orthogonal polynomial test was indicated by a quadratic equation on the effect of VAM dose on the increase in stem height of rosemary $y = 0,1488x^2 + 0,2155x + 12,225$, the highest VAM dose was 20 g/plant with an increase in rosemary stem height of 17,33 cm. the application of VAM at a dose of 20 g/plant produced the highest rosemary plant compared to other doses of treatment. VAM application causes the ability of plant roots to absorb nutrients to increase when compared to without VAM application. This causes more nutrients for plants to be available, thus causing metabolism in growth successful. According to Sari, VAM have a role in increasing the ability of plants to absorb the nutrients needed, so that metabolism for growth can successful (5). The availability of nutrients for cell metabolism causes the apical meristem at the tip of the stem to become actively dividing, resulting in cell elongation and expansion which causes the plant to growth.

In addition, to increase in stem height of rosemary plants did not experience any obstacles in the vegetative growth phase. This is in accordance with the research results of Sunjaya et al, plants that were given VAM treatment experienced an increase in their ability to absorb the nutrients needed, so that the growth process could successful and did not experience obstacles (6). Therefore, the study showed that VAM application had an effect on increasing the height of rosemary plants because in the presence of VAM, the ability of rosemary to absorb nutrients increased resulting in the apical meristem at the tip of the stem actively dividing, resulting in cell elongation which resulted in rosemary growing taller and not stunted.

Mycorrhiza have the most prominent role for their plants, namely high affinity for inorganic phosphate transport, the role of hyphae and

inorganic phosphate polyphosphates that accumulate on the surface of hyphae are converted into protein forms which are then translocated along the mycelium and then enter the host plant tissue (7). Based on this explanation, it can be seen that the application of VAM provides hight affinity for inorganic phosphate, then the polyphosphate that accumulates on the surface of the hyphae turns into protein. Protein in plants has a role in mechanical functions, namely in overall plant growth, especially stem, branches, and leaves. This resulted in the growth of the rosemary plant on the stem cell elongation, resulting in an increase in rosemary height.

2. **Application of Vesicular Arbuscular Mycorrhiza (VAM) Significant Affected on Rosemary Stem Diameter Increase**

The orthogonal polynomial test was shown by a quadratic equation on the effect of VAM dose on the increase in stem diameter of rosemary $y = -0{,}0006x^2 + 0{,}1327x + 0{,}815$, the highest VAM dose was 20 g/plant with an increase in stem diameter of rosemary plants 1,47 mm. the increase in stem diameter was due to the impact of mycorrhiza infection on the roots of rosemary (7). The increase in stem diameter in dicotyledonous plants was closely related to the root system of plants was closely related to the root system of plants formed and with the application of mycorrhiza was also closely related to the level of root infection by mycorrhiza. The occurrence of root infection will form a good root system and then the plant will be able to obtain nutrients and water from the soil optimally, so that good growth and development can be achieved in plants. Rosemary plants will experience an increase in stem diameter due to root infection by VAM which will form a good root system and be able to make plants obtain nutrients and water optimally, especially in increasing rosemary stem diameter.

Application VAM has an impact on increasing and accelerating secondary growth of rosemary plants said that the stem undergoes secondary growth where there is a vascular cambium which is composed of meristematic cells that form a secondary vascular network, dilatation occurs in the cambium, that is cleavage rapidly in a longitudinal and radial direction so that the diameter of the stem will be thicker (8). The growth of rosemary increased due to root infection by VAM, resulting in an increase in the diameter of the rosemary stem due to the vascular cambium of meristematic cells that form secondary tissue. Rosemary stem tissue will divide rapidly, causing the stem diameter to be thicker with VAM application than without VAM.

3. **Application of Vesicular Arbuscular Mycorrhiza (VAM) Significant Affected on Essential Oil Content of Rosemary**

The orthogonal polynomial test is shown by a quadratic equation on the effect of dose. VAM on rosemary essential oil content $y = 0{,}05x^2 + 0{,}3707x + 1{,}2493$ obtained the highest VAM dose of 20 g/plant with essential oil of rosemary 4,38%. VAM has a very significant effect on essential oil content by adding VAM 20 g/plant. This is presumably because the induction of VAM can increase content of secondary metabolites of rosemary plants. The increase in secondary metabolites was caused by the growth of rosemary which also increased so that the nutrient uptake for rosemary

was fulfilled (1). VAM is able to help roots in the absorption of macro and micro elements that are not available, especially phosphate elements. Phosphate elements that are absorbed optimally and adequately will increase the absorption surface, work of phosphatase enzymes, and oxalate enzymes that trigger the formation of secondary metabolites. So that VAM induced rosemary will increase the yield of essential oil secondary metabolites, work of phosphatase enzymes, and oxalate enzymes that trigger the formation of secondary metabolites.

The ability of VAM to increase the yield of secondary metabolites in rosemary is thought to be due to the production of volatile oil metabolites present in rosemary induced by VAM compared to rosemary plants without VAM application. This refers to that there are volatile oil metabolites, namely gingerol, limonene, sesquisabinene, fernesen, and isobornyl acetate which were only identified in herbs introduced by VAM and not found in control plants (2).

VAM functions to increase the ability of plant roots to increase nutrient uptake for plants, it makes the fulfilment of the nutrients needed for primary metabolites, it will increase the yield of plant secondary metabolites. In accordance with the research of that primary metabolites are important factors for plant growth, while secondary metabolites are metabolic products that are not used by plants for growth and are produced more when plants are under stress (9). The fulfilment of primary metabolites will also increase the yield of secondary metabolites.

The main compound resulted from secondary metabolites in the leaves of the rosemary plant according to the research of are carsonic acid, carmosol, and rosmarinic acid. Among the polyphenolic compounds of the flavonoid group, rosmarinic acid has the highest concentration (10). It is suspected that VAM has an effect on rosemary to increase the yield of rosmarinic acid found in capitate gland trichomes. Referring to the research in the genus Coleus, which is a member of the Lamiaceae, volatile metabolites accumulate in special secrety organs, which include capitate, peltate, conoidal, and digitiform gland trichomes (11). Meanwhile, Kasparaviciene et al. stated that volatile secondary metabolites used as drugs such as lipophilic and terpenoid accumulate in special secretory organs, namely trichome capitate in rosemary (12). The increased yield of rosemary secondary metabolites was thought to be due to the secretion of secondary metabolites that occurred in capitate gland trichomes produced more rosmarinic acid with VAM application than control.

CONCLUSION

Based on the results of the research, it can be concluded that:

1. Vesicular Arbuscular Mycorrhiza can be applied to rosemary cultivation.
2. The addition of VAM 20 g/plant resulted in the highest growth at a plant heigh of 17,33 cm and a stem diameter of 1,47 mm.
3. Application of VAM 20 g/plant resulted in the highest essential oil content of 4,38%.

Based on the research that has been done, it can be suggested that:

1. Application of VAM is carried out when the rosemary cuttings have rooted so that they can work optimally on the absorption of nutrients for the plant.

2. It is possible to increase the dose VAM applications for further research.
3. It is necessary to study the metabolite profile of the rosemary essential oil applied by VAM for further research.

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