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Sterilization of Zeolite Media to Increase Arbuscular Mycorrhizal Fungi Colonization in Corn and Sorghum Plants

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ABSTRACT. Productivity of crops such as maize and sorghum is often constrained by low soil fertility, especially in terms of nutrient availability. Arbuscular Mycorrhiza Fungi (FMA) can improve nutrient absorption and support plant growth through a symbiotic relationship with plant roots. However, the success of FMA colonisation is strongly influenced by the quality of the growing medium, which is often contaminated with competing microorganisms that inhibit its effectiveness. To address this issue, this study aimed to evaluate the effect of sterilisation methods on the colonisation of FMA in maize and sorghum plants. The sterilisation methods tested were autoclaving and gamma radiation. This study used a completely randomised design with 8 treatment combinations and 5 replications. Zeolite media was sterilised using autoclaving and gamma radiation at a dose of 50 kGy. Results showed that sterilisation with gamma radiation produced a higher number of mycorrhiza spores and increased root colonisation in maize and sorghum compared to autoclaving. Gamma radiation applied to one sorghum seedling resulted in the highest spore count, while two seedlings in one pot reduced colonisation efficiency due to competition for nutrients. Gamma radiation was more effective in improving the quality of growing media and supporting mycorrhiza colonisation, ultimately enhancing overall plant growth and nutrient absorption.

Keywords : *Gamma Radiation, Mycorrhiza Fungi, Colonisation, Growing Media*

INTRODUCTION

The productivity of crops such as corn and sorghum is often constrained by low soil fertility, especially in terms of nutrient availability and poor soil structure. This problem affects the ability of plants to absorb nutrients, thus inhibiting growth and reducing yields. The use of chemical fertilizers is the main solution, but its application often causes negative environmental impacts, such as water pollution and decreased soil quality (Kalamulla et al. 2022). Therefore, sustainable approaches such as the use of soil microorganisms, especially Arbuscular Mycorrhizal Fungi (AMF), are of important concern in modern agricultural practices (Benaffari et al. 2022).

Therefore, Arbuscular Mycorrhizal Fungi (AMF) are one of the biological solutions that can help this problem. Mycorrhiza are soil microorganisms that work together with plant roots to increase nutrient absorption, especially phosphorus and nitrogen. Mycorrhiza can also help plants survive difficult conditions, such as drought, salinity, and pest attacks (Diagne et al. 2020). In addition, mycorrhiza also improves soil by increasing the activity of other microbes and improving soil structure (Makarian et al. 2016).

However, the success of AMF utilization is highly dependent on the quality of the growing media used. Unsterile growing media often contain competing microorganisms that can inhibit mycorrhiza colonization of plant roots, thereby reducing its effectiveness (Saia et al. 2015). For this reason, media sterilization techniques, such as autoclaving and gamma radiation, have been used to reduce unwanted contamination. The combination of media sterilization techniques and mycorrhiza inoculation has been shown to increase the number of spores, the degree of root infection, and the efficiency of plant nutrient uptake (Hassena et al. 2022).

Among the various sterilization methods, gamma radiation has several advantages over autoclaving. Gamma radiation is able to penetrate the material thoroughly, even in media with thick packaging, thus ensuring more efficient elimination of microorganisms than autoclaving (Sparks 1984). In addition, this process does not require high heat or pressure, thus minimizing the risk of degradation of media structures such as soil or zeolite (Mcnamara et al. 2003). With the ability to produce a sterile environment without leaving harmful chemical residues, gamma radiation is a more environmentally friendly method than conventional techniques such as autoclaving (Ley et al. 1969).

The advantages of this sterilization method were tested through its application on corn and sorghum plants, two strategic food crops that require high nutrients. In this study, zeolite-based planting media that had been sterilized by autoclaving and gamma radiation were tested for their effectiveness in mycorrhiza colonization. Parameters such as the number of spores, percentage of root infection, plant height, and root dry weight were observed to evaluate the effectiveness of each sterilization method (Delavaux et.al. 2017).

This study aims to evaluate the effect of planting media sterilization methods on the effectiveness of mycorrhiza in supporting the growth of corn and sorghum plants. The results are expected to provide a sustainable solution to increase plant productivity while reducing dependence on chemical fertilizers. In addition, this study contributes to the development of environmentally friendly methods in modern agricultural practices (Kalamulla et al. 2022)

MATERIALS AND METHODS

Tools and Materials.

This study used several main tools to support each stage of the experiment. An autoclave was used to sterilize the zeolite media at high temperatures, while a gamma irradiator was used to sterilize the media with a radiation dose of 50 kGy. A 250 ml plastic pot was prepared as a container for the planting media and test plants. A graduated sieve with a pore size of 355 µm, 250 µm, 125 µm, and 45 µm was used for the isolation of Arbuscular Mycorrhizal Fungi (AMF) spores, while a microscope was used to observe AMF spores and the level of root infection in plants. The materials used include zeolite media as the main component of the planting media, rice husk charcoal, and peat as additional materials. A 5% NaOCl solution was used to sterilize seeds before sowing. In addition, NPK fertilizer (Hyponex 25-5-20) was applied to support plant growth during maintenance. Corn and sorghum seeds were selected as test plants, and AMF inoculants in the form of spores from the genus Glomus sp. and Gigaspora sp. which are collections of the Soil Science Laboratory of Padjadjaran University (UNPAD) were used in each inoculation treatment.

Experimental Design.

The experimental design used was a Completely Randomized Design (CRD), with 8 treatment combinations and 5 replications for each treatment, so that there were a total of 40 experimental pots. This research was conducted in the Plant Fertilization and Nutrition greenhouse, National Nuclear Energy Agency (BATAN). This experiment involved variations in zeolite media sterilized by autoclave or radiation, corn and sorghum plants, and different numbers of seeds in each pot. An explanation of the treatment combinations in this experimental design is presented in Table 1 below:

Table 1. Treatment Combinations in Experimental Design

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No	Treatment Code	Treatment Description
1.	ZAJ1	Autoclave Zeolite + 1 corn seedling
2.	ZRJ1	Radiation Zeolite + 1 corn seedling
3.	ZAS1	Autoclave Zeolite + 1 sorghum seedling
4.	ZRS1	Radiation Zeolite + 1 sorghum seedling
5.	ZAJ2	Autoclave Zeolite + 2 corn seedling
6.	ZRJ2	Radiation Zeolite + 2 corn seedling
7.	ZAS2	Autoclave Zeolite + 2 sorghum seedling
8.	ZRS2	Radiation Zeolite + 2 sorghum seedling

Research Implementation

Preparation of Growing Media

This research was conducted by following the stages that had been designed based on the method of (Mansur et al. 2012). The growing media used in this study were zeolite, rice husk charcoal, peat as much as 200 grams/pot. The media was washed until clean then put into a heat-resistant plastic bag then sterilized using radiation of 50 kGy and sterilized with an autoclave at a temperature of 120°C for 2 hours. The 250 ml plastic pot was filled according to the treatment.

Preparation of AMF Isolates

The mycorrhiza isolates used originated from the Soil Biology Laboratory collection, Faculty of Agriculture, Universitas Padjadjaran (Unpad), Faculty of Agriculture, UNPAD. The mycorrhiza spores were isolated using a graduated sieve measuring 355, 250, 125, 45 µm (wet filtration method). The spores filtered on each sieve size were observed under a microscope, and then separated based on their genus, namely *Glomus sp*. and *Gigaspora sp.*

Seed Preparation

Seed preparation begins with a germination test to determine the number of seeds that are able to germinate well. Sorghum seeds are soaked in a 5% NaOCl solution for 2 minutes according to the method, then rinsed thoroughly using water. The cleaned seeds are sown in a germination tray using clean and sterile zeolite media. The sorghum seed germination process takes 3–4 days.

Planting

Pots that have been filled with media according to the treatment are given fresh mycorrhiza inoculant in the form of 10 spores which are attached directly to the roots of the sorghum seedlings. After that, the pot is closed again using the media until a distance of about 1 cm is left from the top of the pot. Two sorghum seedlings are inserted into the planting hole ±1 cm deep from the surface of the media. Furthermore, the media is watered with sufficient water, then the pot is stored in a greenhouse according to the previously randomized layout.

Maintenance

Plant maintenance is carried out by watering the planting media every day using clean water. Fertilization was carried out two weeks after the pot culture was planted, using NPK fertilizer (Hyponex) with low phosphorus content (25-5-20). Fertilization was repeated twice a week for 2.5–3 months. Watering was adjusted to the field capacity of each growing medium. Propagule development was observed during the maximum vegetative period, namely when the plants were 12 MST (Weeks After Planting).

Observations

Observations were carried out to assess the effectiveness of the treatment on the plants. The parameters observed included the number of spores and the degree of root infection as indicators of mycorrhiza colonization. In addition, plant height and root dry weight were measured to determine the effect of treatment on plant growth.

Harvest

Harvesting was carried out after the sorghum plants were 2.5–3 months old. Watering and fertilization were stopped two weeks before harvest for the drying process (stressing). After the plants were dry, the crown of the plant was cut starting from the base of the root. The root part was separated, washed clean using running water, and weighed to measure the fresh weight of the roots. The roots were then cut into pieces for further observation, such as the number of spores, percentage of root infection, and weight of the host plant roots.

RESULTS AND DISCUSSION

Observation of plant height in sorghum and corn plants

Observation of plant height in corn and sorghum plants showed significant variation in growth among treatments. In general, corn plants tend to have a greater height compared to sorghum plants in each treatment. This is thought to be due to the genetic nature of corn which has the ability to grow vegetatively faster and more efficiently in utilizing resources such as nutrients and light (Smith and Read 2008). In addition to genetic factors, plant age also affects yields, because at the age of 4 to 12 weeks, corn grows faster than sorghum. Therefore, the interaction between genetic factors, media, and plant age greatly affects the observed plant height.

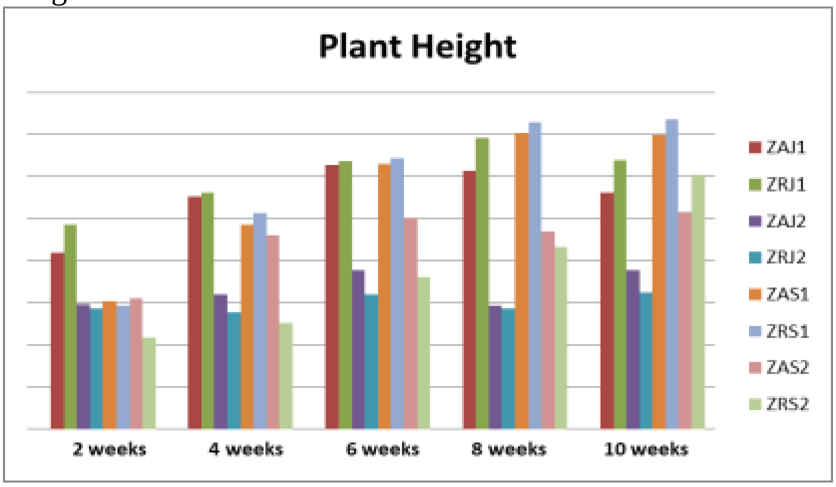

Figure 1. Effect of Media Sterilization on Corn and Sorghum Plant Height

Treatment ZRS1 (zeolite sterilized with gamma radiation and one sorghum seedling) produced the highest sorghum plant height, while treatment ZRJ1 (zeolite sterilized with gamma radiation and one corn seedling) gave the best results in corn plants. This is likely due to gamma radiation increasing the availability of nutrients in the growing medium and reducing competing microorganisms, thus providing a better environment for plant growth. This is in line with Zhang et al. (2016), who reported that gamma radiation increases the availability of carbon and nitrogen by eliminating soil fauna, as well as reducing competing microorganisms such as saprophytic fungi and protozoa, thus supporting a more optimal growing environment. In contrast, treatments ZAS2 and ZAJ2 using autoclave produced the lowest plant height, possibly due to competition between seedlings that occurred in smaller pots. Thus, the number of seedlings and the type of media sterilization had a significant impact on plant growth.

Overall, the results of this study indicate that media sterilization plays an important role in supporting plant growth. Gamma radiation proved to be more effective than autoclave in increasing the availability of nutrients in the growing medium. Therefore, the use of gamma radiation can be recommended to increase plant growth efficiency through optimization of the quality of the planting medium.

Table 2. Effect of Zeolite Media Sterilization on Root Dry Weight in Corn and Sorghum Plants

Treatment	Average
ZRS2 (Radiated zeolite + 2 sorghum seedlings)	0.390 a
ZRJ2 (Radiated zeolite + 2 corn seedlings)	0.425 a
ZAJ2 (Autoclaved zeolite + 2 corn seedlings)	0.632 a
ZAS1 (Autoclaved zeolite + 1 sorghum seedling)	0.717 ab
ZAJ1 (Autoclaved zeolite + 1 corn seedling)	1.020 bc
ZAS2 (Autoclaved zeolite + 2 sorghum seedlings)	1.039 bc
ZRS1 (Radiated zeolite + 1 sorghum seedling)	1.054 bc
ZRJ1 (Radiated zeolite + 1 corn seedling)	1.074 c

Percentage of root infection degree

The degree of root colonization by Arbuscular Mycorrhizal Fungi (AMF) reflects the success of the symbiosis between plants and fungi, which plays a role in the absorption of phosphorus (P) and nitrogen (N), and increases tolerance to environmental stress. This colonization also involves the exchange of carbon from plants to fungi (Campo et al. 2020). However, the effectiveness of this colonization is influenced by various external factors, such as plant type, soil conditions, the presence of other microorganisms, and treatment of the planting medium. Sterilization methods, such as autoclaving or gamma radiation, can change the physical, chemical, and biological properties of the soil, which ultimately affect the symbiotic relationship formed between plants and fungi (Hasanain et al. 2014). These factors, especially the influence of radiation media, provide varying results in supporting mycorrhiza colonization, as seen in the results of this study. Thus, addressing these influencing factors is crucial for optimizing the symbiotic benefits of mycorrhiza in agricultural practices.

Table 3. Effect of Zeolite Media Sterilization on Arbuscular Mycorrhizal Fungi Colonization in Corn and Sorghum Plants

Treatment	Percentage (%)	Category
ZAJ1 (Autoclave Zeolite + 1 corn seedling)	4.37 ± 10.64	Low
ZRJ1 (Radiation Zeolite + 1 corn seedling)	5.58 ± 11.33	Low
ZAJ2 (Autoclave Zeolite + 1 sorghum seedling)	2.80 ± 7.31	Low
ZRJ2 (Radiation Zeolite + 1 sorghum seedling)	11.40 ± 19.33	Medium
ZAS1 (Autoclave Zeolite + 2 corn seedling)	2.36 ± 8.33	Low
ZRS1 (Radiation Zeolite + 2 corn seedling)	23.57 ± 19.99	Medium
ZAS2 (Autoclave Zeolite + 2 sorghum seedling)	1.92 ± 4.44	Low
ZRS2 (Radiation Zeolite + 2 sorghum seedling)	1.65 ± 2.17	Low

Based on the research results, the ZRJ2 treatment (Zeolite radiation + mycorrhizal inoculant, 2 corn seedlings) showed the highest degree of colonization, which was 19.33% ± 11.40. According to the root colonization degree assessment category according to O'Connor et al. (2001), this value is included in the medium category (10-30%). This is likely due to the influence of radiation which improves the quality of the media and increases the availability of nutrients, thus supporting mycorrhiza colonization. Although a high level of root colonization can indicate the success of mycorrhizal inoculation, however, this is not always directly related to increased plant growth. According to Powell and Bagyaraj (1984), colonization of the roots of plants inoculated with mycorrhiza is not always closely related to the effectiveness of mycorrhiza in increasing plant yields. Corn plants support colonization more than sorghum because corn root exudates provide carbohydrate compounds and amino acids needed for the growth of mycorrhiza hyphae (Smith and Read 2008).

However, despite high colonization, the effectiveness of mycorrhiza in supporting plant growth still needs to be analyzed to understand its practical benefits. The ZRJ2 treatment proved that the combination of radiation media with corn plants provided optimal results in supporting mycorrhiza colonization. Radiation media increased colonization efficiency, while the nature of corn plants that support mycorrhiza growth maximized the success of this symbiont interaction. This study underlines that high colonization does not always correlate directly with increased plant growth, as explained by Powell and Bagyaraj (1984). Further research is needed to optimize inoculation conditions and ensure more consistent colonization results for application in sustainable agricultural practices.

Spore count

The number of spores is an important indicator in assessing the success of colonization of Arbuscular Mycorrhizal Fungi (AMF) in planting media. In this study, the treatment of media sterilization using gamma radiation compared to autoclaving showed significant results on the number of spores produced. Sterilization with gamma radiation effectively reduces competing microorganisms and improves the quality of the planting media, providing a more optimal environment for the growth and colonization of AMF. Figure 2 shows the structure of vesicles and hyphae in plant roots that support AMF colonization results. This is in line with research (Budi 2012) showing that sterilization of planting media can reduce competing microorganisms, thereby supporting mycorrhizal colonization and significantly increasing plant growth. In addition, research (Nurrobifahmi et.al 2017) found that gamma radiation at a certain dose was more effective than autoclaving in suppressing contaminant microbes in carrier materials, supporting the viability of Gigaspora margarita spores, and creating a more conducive planting media environment for mycorrhizal growth

Table 4. Effect of Zeolite Media Sterilization on the Average Number of Arbuscular Mycorrhizal Fungi Spores in Corn and Sorghum Plants

Treatment	Average
ZAJ1 (Autoclave Zeolite + 1 corn seedling)	3.66
ZRJ1 (Radiation Zeolite + 1 corn seedling)	1.40
ZAJ2 (Autoclave Zeolite + 1 sorghum seedling)	1.66
ZRJ2 (Radiation Zeolite + 1 sorghum seedling)	3
ZAS1 (Autoclave Zeolite + 2 corn seedling)	2.5
ZRS1 (Radiation Zeolite + 2 corn seedling)	6
ZAS2 (Autoclave Zeolite + 2 sorghum seedling)	1.33
ZRS2 (Radiation Zeolite + 2 sorghum seedling)	1

Based on the data in table 4, the highest average number of spores was found in the ZRS1 treatment (gamma radiation zeolite, 1 sorghum seedling) with 6 spores. This is likely due to the effect of gamma radiation which creates a sterile medium and increases the availability of nutrients, thus supporting the colonization and development of mycorrhizal spores to the maximum. In addition, the use of one sorghum seedling reduces competition between plants in utilizing media nutrients, providing more space for mycorrhizal growth. Conversely, the lowest average number of spores was found in the ZRS2 treatment (gamma radiation zeolite, 2 sorghum seedlings) with 1 spore. This is likely due to nutrient competition between two sorghum seedlings planted in one pot, which reduces the contribution of root exudates to support mycorrhizal growth. Less root exudates inhibit the development of mycorrhizal spores. This finding is supported by (Smith and Read 2008), which states that mycorrhizal fungi are completely dependent on organic carbon from host plant root exudates, and the amount of exudates greatly influences the development and colonization of mycorrhizal hyphae. The results showed that the method of sterilization of the planting medium affected the number of spores produced, with gamma radiation producing more optimal conditions than autoclaving. However, the number of seedlings in one pot also affected the success of colonization. Further research is needed to evaluate the relationship between the number of spores and the effectiveness of mycorrhizae in supporting overall plant growth.

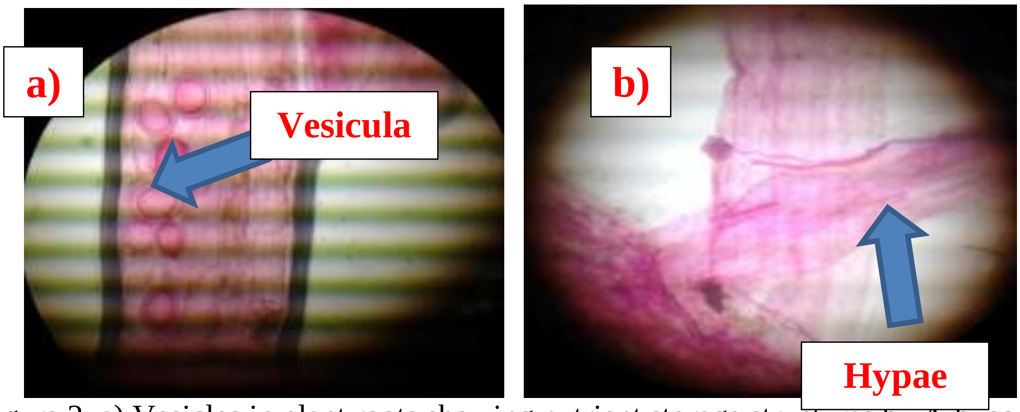

Figure 2. a) Vesicles in plant roots showing nutrient storage structures by Arbuscular Mycorrhizal Fungi (AMF), Hyphae of Arbuscular Mycorrhizal Fungi (AMF)

CONCLUSION

Sterilization of planting media using gamma radiation has a positive effect on plant growth, with a significant increase in plant height, root weight, percentage of root infection colonies, and the number of Arbuscular Mycorrhizal Fungi (AMF) spores compared to sterilization using an autoclave. Optimal planting media conditions after gamma radiation sterilization support more effective AMF colonization on plant roots, as indicated by the well-observed vesicle and hyphae structures. The application of gamma radiation sterilization methods in agricultural research and practice can be an option, especially in planting media used to optimize the symbiotic relationship of AMF with plants, and further research is needed to evaluate the optimal dose of gamma radiation in various planting media conditions.

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REFERENCES

Budi, Sri Wilarso. 2012. “Pengaruh Sterilisasi Media Dan Dosis Inokulum Terhadap Pembentukan Ektomikoriza Dan Pertumbuhan Shorea Selanica Blume.” *Jurnal Silvikultur Tropika* 03 (02): 76–80.

Camille S. Delavaux, Lauren M. Smith-Ramesh, Sara E. Kuebbing. 2017. “Beyond Nutrients: A Meta-Analysis of the Diverse Effects of Arbuscular Mycorrhizal Fungi on Plants and Soils.” *Ecology* 98 (8): 2111–19. https://doi.org/10.1111/ijlh.12426.

Campo, Sonia, Héctor Martín-cardoso, Marta Olivé, Eva Pla, Mar Catala-forner, Maite Martínez-eixarch, and Blanca San Segundo. 2020. “Effect of Root Colonization by

Arbuscular Mycorrhizal Fungi on Growth , Productivity and Blast Resistance in Rice." *Rice* 13 (42): 1–14.

Diagne, Nathalie, Mariama Ngom, Pape Ibrahima Djighaly, and Dioumacor Fall. n.d. "Roles of Arbuscular Mycorrhizal Fungi on Plant Growth and Performance : Importance in Biotic And."

F.J. Ley, J. Bleby, Coates, Marie E, J.S. Paterson. 1969. "Sterilization Of Laboratory Animal Diets Using Gamma Radiation." *Lab Anim* 3: 221–54.

Hasanain, Fatima, Katharina Guenther, Wayne M Mullett, Fatima Hasanain, Katharina Guenther, Wayne M Mullett, and Emily Craven. 2014. "Gamma Sterilization of Pharmaceuticals —— A Review of the Irradiation of Excipients , Active Pharmaceutical Ingredients , and Final Drug Product Formulations Gamma Sterilization of Pharmaceuticals — A Review of the Irradiation of Excipients , Active Pharmaceutical Ingredients ,." https://doi.org/10.5731/pdajpst.2014.00955.

Hassan Makarian, Vahid Poozesh, Hamid Reza Asghari & Meisam Nazari. 2016. "Interaction Effects of Arbuscular Mycorrhiza Fungi and Soil Applied Herbicides on Plant Growth." *Communications in Soil Science and Plant Analysis* 3624 (February): 1–30. https://doi.org/10.1080/00103624.2016.1146744.

Hassena, Ameni Ben, Mohamed Zouari, Wahid Khabou, and Nacim Zouari. 2022. "Impact of Inoculation with Single and Mixed Species of Arbuscular Mycorrhizal Fungi on the Soil Fertility and the Nutrient Uptake of Young Olive Plants," no. September: 48–54.

Kalamulla, Ruwanthika, Samantha C Karunarathna, Saowaluck Tibpromma, Mahesh C A Galappaththi, Nakarin Suwannarach, Steven L Stephenson, Suhail Asad, Ziad Salman Salem, and Neelamanie Yapa. 2022. "Arbuscular Mycorrhizal Fungi in Sustainable Agriculture." *Sustainability*, 1–14.

Mansur, Irdika, Abimanyu Dipo Nusantara, Rr. Yudhy Harini Bertham. 2012. *Bekerja Dengan Fungi Mikoriza*. Edited by Nia Januarini. Cetakan Pe. Bogor, Indonesia: SEAMEO BIOTROP (.

Mcnamara, N P, H I J Black, N A Beresford, and N R Parekh. 2003. "Effects of Acute Gamma Irradiation on Chemical , Physical and Biological Properties of Soils." *Applied Soil Ecology* 24: 117–32. https://doi.org/10.1016/S0929-1393(03)00073-8.

Nurrobifahmi, Anas, Iswandi, and Yadi Setiadi. 2017. "Pengaruh Metode Sterilisasi Radiasi Sinar Gamma Co-60 Dan Autoklaf Terhadap Bahan Pembawa , Viabilitas Spora Gigaspora Margarita Dan Ketersediaan Fe , Mn , Dan Zn Effect of Sterilization Using Gamma Rays and Autoclave on Carrier Materials , Viability of S." *Jurnal Tanah Dan Iklim* 41 (1): 1–8.

Saia, Sergio, Paolo Ruisi, Veronica Fileccia, Giuseppe Di Miceli, Gaetano Amato, and Federico Martinelli. 2015. "Metabolomics Suggests That Soil Inoculation with Arbuscular Mycorrhizal Fungi Decreased Free Amino Acid Content in Roots of Durum Wheat Grown under N-Limited , P-Rich Field Conditions," 1–15. https://doi.org/10.1371/journal.pone.0129591.

Sally E. Smith, David J. Read. 2008. *Mycorrhizal Symbiosis (3rd Ed.)*. (3rd Editi. London: Academic Press (Elsevier).

Sparks, Beverly J. 1984. "Product Sterilization." *AORN JOURNAL* 40 (3): 388–90.

Zhang, Shixiu, Shuyan Cui, Xiaoming Gong, Liang Chang, Shuxia Jia, Xueming Yang, Donghui Wu, and Xiaoping Zhang. 2016. "Effects of Gamma Irradiation on Soil Biological Communities and C and N Pools in a Clay Loam Soil." *Applied Soil Ecology* 108: 352–60. https://doi.org/10.1016/j.apsoil.2016.09.007.