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The effect of botanical pesticide application and mycorrhiza on colonization of mycorrhiza, soil microbial communities, growth and yield of maize on Ultisols

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Abstract

An experiment to study the effect of synthetic and botanical pesticide and arbuscular mycorrhizae (AMF) on mycorrhizal colonization, population of soil microbes, growth and yield of maize on Ultisols was carried out in green house of Agriculture Faculty, Universitas Padjadjaran at Jatinangor, Sumedang District, West Java Indonesia

The experiment design used was Factorial Randomized Block, consisted two factors. The first factor was pesticide consisted five levels i.e. without pesticide; neem (*Azadirachta indica* A. Juss) pesticide; *Annona muricata* pesticide ; furadan and regent. The second factor was mycorrhiza consisted two levels i.e. without AMF inoculation and AMF inoculation. The experiment consisted two units with three replications; the first unit was harvested in the end of vegetative period to observe percentage of mycorrhizal infection, population of soil bacteria and fungi, fosfor uptake and dry weight of shoot, and the second unit was harvested in the end of generative period to find out the yield of maize.

The results of this experiment showed that there was no interaction effect between pesticide and AMF on percentage of mycorrhizal infection, population of soil bacteria and fungi, fosfor uptake, dry weight of shoot and yield of maize. Pesticide decreased percentage of mycorrhizal infection and population of soil bacteria and increased yield of maize. Furthermore, AMF increased fosfor uptake and yield of maize.

Keywords: Pesticide; AMF; Community of soil microorganism; Growth

1. Introduction

Pest and disease attacks on food crops are one of the obstacles that can cause a decrease in crop yields, even the worst consequence is crop failure. The use of synthetic (chemical) pesticides in controlling this disease is something that is often done by farmers in Indonesia. The use of synthetic pesticides in overcoming this problem often has negative impacts, including the emergence of disease resistance, environmental pollution, and ingested pesticide residues that can endanger human health [1]. To avoid these detrimental side effects, it is necessary to look for alternative ingredients that can replace or reduce the use of synthetic pesticides. One alternative that can be studied and researched is the use of botanical ingredients derived from plants in Indonesia, especially medicinal plants. From the research results of Suharti et al. [2] it is known that extracts of several types of medicinal plants in Indonesia (in tropical areas) can inhibit the growth of several pathogenic fungi on food plants.

An agricultural system that relies heavily on high inputs in the form of energy for tillage, as well as fertilizers and other agrochemicals will continuously cause residual effects that affect the diversity of soil biota communities [3]. Pesticides, therefore, also influence soil microbial communities including organisms that engage in mutualistic plant symbioses that play a crucial role in its mineral nutrition, such as arbuscular mycorrhizal fungi [4]. Furthermore, as a consequence

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of the effects of decreasing the diversity of soil biota communities and even the loss of certain types and species, it is very likely that this will cause the loss of the function and role of these microbes.

To make efficient use of artificial fertilizers, agricultural biotechnology is now important in utilizing microbes that play a role in nutrient transfer to plants. To increase nutrient absorption, especially P, it can be done by utilizing the symbiosis between plant root functions, namely arbuscular vesicular mycorrhiza (AMF). According to Smith et al [5], several studies have proven that P absorption by plants can be increased by the presence of mycorrhizal infection in plant roots. AMF is known to increase the ability of plants to absorb nutrients and water, increase plant resistance to drought, protect plants from poisoning by heavy metals and attack by root pathogens, and help plant growth in unfavorable soil conditions [6], [7].

Apart from being able to control disease, the use of pesticides can actually affect the development of mycorrhiza. The use of the fungicide carboxin, vitavax, can significantly reduce *Glomus deserticola* mycorrhizal colonization. The use of the fungicides benomyl, calixin, topsin and banrof can actually reduce mycorrhizal infections [8]. For integrated control of pests and diseases, environmentally friendly control alternatives are needed, can be made by farmers using materials available in nature, and are cheap. One effective and efficient alternative for controlling pests and diseases is to use pesticides derived from plants, known as botanical pesticides [9].

Botanical pesticides are safer than chemical pesticides, thereby ensuring the preservation of environmental resources. Much evidence shows that plants are a storehouse of chemicals called secondary metabolite production which are used to protect themselves from various plant pests [10]. Plants used as botanical pesticides can include seed or rhizome powder, leaf, seed or rhizome extracts and essential oils from flowers or leaf litter.

Neem is an annual plant that contains the main active ingredients azadirachtin, meliantriol, salanin, nimbin, nimbidin and other substances which are able to control around 127 types of pests, including insect pests, nematodes, fungi and others [11]. The neem tree is only known as a shade and protective tree, whereas in fact the neem tree has great potential to be used as a plant pest medicine (pesticide). Researchers have found that the secondary metabolites contained in the neem tree function as insecticide (anti-insect), bactericide (anti-bacterial), miticide (anti-mite), viricidal (anti-virus), ovicidal (prevents egg cell division), spermaticial (causes sperm infertility), rodenticide (anti-rodent), fungicidal (anti-fungal), antimalarial, nematicide (anti-worm), nitrogen release inhibitor, and cosmetic ingredient (as neem oil) [12]. However, currently what has been well proven in the field is the effectiveness of neem as an insecticide [13].

To study the effect of pesticides on mycorrhiza and other soil microorganisms, it is necessary to carry out research using both synthetic and botanical pesticides combined with the application of mycorrhiza to food plants, in this case maize plants, which are food plants that can be in symbiosis with mycorrhiza and can be attacked by disease.

2. Materials and methods

The research was carried out at plastic houses located in Cibiru District, Bandung, West Java Province, Indonesia. The design used was a factorial randomized block design with the first factor was pesticide (P) consisting of five levels (no pesticide; botanical pesticide from neem seeds; botanical pesticide from soursop seeds; synthetic pesticide (Furadan) ; synthetic pesticide (Regent). The second factor is mycorrhiza (M) *Glomus* sp. , which consists of two levels (without mycorrhizal inoculation and with mycorrhizal inoculation 10 g/polybag)

The main observations in this research were percentage of root infection by mycorrhiza with the root staining technique from Kormanik & McGraw [14], total population of soil bacteria and soil fungi using using the dilution plate method , plant phosphorus uptake determined by the Kjeldahl method, and dry weight of plants and maize crop yields that was observed at the end of vegetative phase.

3. Results and discussion

3.1. Mycorrhizal Infection

The statistical analysis showed that there was no interaction effect between pesticide application (synthetic and botanical pesticides) and AMF on the degree of mycorrhizal infection. The independent effects of pesticide treatment and AMF can be seen in Table 1. The application of pesticides has a significant effect on reducing the degree of mycorrhizal infection. This is in line with the results of research by He et al. [15] which states that the use of fungicides can reduce mycorrhizal colonization and infection.

In this table it can also be seen that the application of botanical pesticides compared to synthetic pesticides does not significantly differ in the degree of mycorrhizal infection. It is suspected that there are differences in targets or application methods in the use of synthetic and botanical pesticides so that there is no real difference between the application of biological pesticides and botanical pesticides on the degree of mycorrhizal infection. However, it can be clearly seen that the application of synthetic pesticides compared to botanical pesticides reduces the degree of mycorrhizal infection.

Table 1 Effect of pesticides (synthetic and botanical pesticides) and AMF on the degree of mycorrhizal infection

Treatments	**Mycorrhizal Infection (%)**
Pesticide	
No pesticide	52.17 b
Neem pesticide	39.33 a
Soursop pesticide	38.33 a
Furadan	35.11 a
Regent	34.28 a
AMF	
No AMF	37.53 a
With AMF	37.53 a

Note: Numbers followed by the same letter are not significantly different according to Duncan's test at the 5% level

In Table 1 it can be seen that the application of mycorrhiza does not have a significant effect on increasing the degree of mycorrhizal infection. It is suspected that there is an influence of indigenous mycorrhizal spores in the Ultisols soil so that the effect of the mycorrhizal inoculum is not significant on the degree of mycorrhizal infection.

3.2. Soil Bacterial Population

The statistical test results showed that there was no interaction between pesticide application and AMF on the population of soil bacteria. From Table 2 it can be seen that pesticide application has a significant effect on the population of soil bacteria. In this study, it was found that the application of plant-based pesticides was significantly different from synthetic pesticides in influencing the population of soil bacteria. The application of synthetic pesticides apparently reduces the total population of soil bacteria of maize plants. Meanwhile, the application of neem pesticide actually significantly increased the population of soil bacteria. It is suspected that the pesticide made from neem seeds does not contain compounds/substances that are antagonistic to bacterial growth in general.

Table 2 Effect of pesticides (synthetic and botanical pesticides) and AMF on the population of soil bacteria

Treatments	**Soil bacterial population (10^7 CFU g^{-1} soil)**
Pesticide	
No pesticide	5.38 ab
Neem pesticide	6.19 c
Soursop pesticide	5.45 b
Furadan	4.33 a
Regent	4.55 a
AMF	

No AMF	5.39 a
With AMF	5.39 a

Note: Numbers followed by the same letter are not significantly different according to Duncan's test at the 5% level

The results of statistical tests showed that applying AMF to maize plants did not affect the population of soil bacteria (Table 2). It is suspected that there is an influence of indigenous mycorrhiza which can influence the activity of soil bacteria. Therefore, this could mean that the mycorrhiza inoculum provided does not significantly affect the activity of soil bacteria.

3.3. Population of Soil Fungi

The results of statistical tests showed that there was no interaction effect between pesticide application and AMF on the population of soil fungi on Ultisols. In Table 3 we can see the independent influence of pesticide treatment (synthetic and botanical) and AMF on the population of soil fungi.

The effects of synthetic pesticides and plant-based pesticides do not have a significant effect on the total population of soil fungi. This can be seen in Table 3, which shown that each pesticide treatment, both synthetic and botanical, did not affect the population of soil fungi. It is suspected that the growth cycle of soil fungi has stabilized at the end of vegetative period of the plant. This means that the activity of soil fungi at this time is not influenced by external factors because there has been a balance with their habitat/environment. If the calculation of the number of soil fungal populations is carried out before the final vegetative phase, it is possible that there will be a real influence between synthetic and botanical pesticide treatments.

Table 3 Effect of pesticides (synthetic and botanical pesticides) and AMF on soil fungi populations

Treatments	**Soil fungi population (10^6 CFU g^{-1} soil)**
Pesticide	
No pesticide	3.97 a
Neem pesticide	3.35 a
Soursop pesticide	4.43 a
Furadan	4.30 a
Regent	3.93 a
AMF	
No AMF	3.85 a
With AMF	4.14 a

Note: Numbers followed by the same letter are not significantly different according to Duncan's test at the 5% level

In Table 3 it can be seen that the statistical test results show that the independent effect of AMF treatment did not have a significant influence on the total number of soil fungi. This is thought to be because the Ultisols soil used in this study was not sterilized so the AMF inoculant given did not have a real effect. However, in Figure 3 it can be seen that giving AMF to maize plants treated with soursop, furadan and regent pesticides was able to increase the total number of soil fungi.

3.4. Plant P Uptake

The results of statistical tests showed that there was no interaction effect between pesticide application and AMF on P uptake of maize plants in the late vegetative phase. In Table 4 we can see the independent effects of pesticide treatment (synthetic and botanical) and AMF on the P uptake of maize plants.

Table 4 Effect of pesticides (synthetic and botanical pesticides) and AMF on phosphorus uptake

Treatments	**Plant P Uptake (mg kg^{-1})**
Pesticide	
No pesticide	34.90 a
Neem pesticide	42.40 a
Soursop pesticide	37.98 a
Furadan	34.21 a
Regent	29.41 a
AMF	
No AMF	31.77 a
With AMF	39.77 a

Note: Numbers followed by the same letter are not significantly different according to Duncan's test at the 5% level

This research showed that the application of synthetic and botanical pesticides does not have a real effect on the phosphorus uptake of maize plants. In this study, there was no significant difference between synthetic pesticide treatments compared to botanical pesticides on phosphorus uptake. This is thought to be related to the application techniques and doses which are not the same so that the effect of these two pesticides is not significantly different on phosphorus nutrient uptake.

In Table 4 it can be seen that AMF inoculation can significantly increase the phosphorus uptake of maize plants in the late vegetative phase. This is due to the ability of mycorrhiza to absorb P nutrients from the soil which is far from plant roots. An increase in P absorption capacity by mycorrhizal plants can occur directly through the external hyphae system and indirectly due to changes in root physiology. The network of external hyphae expands the absorption surface area further to search for nutrients that are relatively inaccessible to the root system [16], [17].

3.5. Plant Dry Weight

The statistical test results showed that there was no interaction between pesticide application (synthetic and botanical) and AMF on the dry weight of maize plants. The results of this research showed that the application of synthetic and botanical pesticides does not have a significant effect on the dry weight of maize plants (Table 5).

Table 5 Effect of pesticides (synthetic and botanical pesticides) and AMF on dry weight of maize

Treatments	**Plant P Uptake (g plant^{-1})**
Pesticide	
No pesticide	11.15 a
Neem pesticide	13.57 a
Soursop pesticide	11.52 a
Furadan	12.02 a
Regent	9.96 a
AMF	
No AMF	11.17 a
With AMF	12.12 a

Note: Numbers followed by the same letter are not significantly different according to Duncan's test at the 5% level

However, in Figure 6 it can be seen that the synthetic pesticide (regent) provides the lowest dry weight of maize plants compared to other treatments. The dry weight of plants was not influenced by pesticide application, this is because in this study it turned out that pesticides did not affect nutrient uptake (in this case phosphorus). Because nutrient uptake is not affected, the dry weight of maize plants is not affected by pesticide application.

In Table 5 it can be shown that AMF application does not affect the increase in dry weight of maize plants. Even though phosphorus nutrient uptake increased due to AMF inoculation, it turned out that plant dry weight was not affected by AMF inoculation. It is suspected that plant dry weight is not only influenced by phosphorus uptake, but is influenced by many other nutrients which were not analyzed in this study.

However, from data in Table 5 it can be seen that the plants inoculated with AMF showed a higher dry weight of maize plants. This shows that AMF inoculation was able to increase better plant growth as indicated by higher plant dry weight compared to plants without mycorrhizal inoculation. The application of mycorrhiza can increase plant growth due to increased plant nutrient uptake [18].

3.6. Yead of Maize

The results of statistical tests showed that there was no interaction between pesticide treatment and AMF on maize yields. The independent effects of pesticide treatment (synthetic and botanical) and AMF on maize crop yields, in this case dry maize kernel weight, can be seen in Table 6.

Table 6 Effect of pesticides (synthetic and botanical pesticides) and AMF on yield of maize

Treatments	Yoeld of Maize (g)
Pesticide	
No pesticide	11.57 a
Neem pesticide	13.84 b
Soursop pesticide	13.14 b
Furadan	13.27 b
Regent	12.30 ab
AMF	
No AMF	12.08 a
With AMF	13.58 b

Note: Numbers followed by the same letter are not significantly different according to Duncan's test at the 5% level

In Table 6 it can be seen that pesticide application significantly affects maize yieldb (dry shell weight of maize). From this research it was found that the use of both synthetic and botanical pesticides did not reduce maize yields, in fact there was an increase. This can be expected because the dose of pesticide used is in accordance with the recommendations so that it is able to control pests that disturb maize plants so that the yield of maize plants is higher than plants that are not treated with pesticides.

From Table 6 it can be seen that AMF inoculation is able to increase maize crop yields. Wahab et al. [19] stated that AMF plays a role in the absorption of macro and micro nutrients, increases resistance to drought, protects plants from poisoning by heavy metals, protects roots from pathogen attacks, and helps plant growth in less favorable soil conditions. In plants inoculated with AMF, the nutrient content is higher than in plants not inoculated with AMF [20]. Therefore, plants inoculated with AMF give better crop yields than plants that are not inoculated.

4. Conclusion

There was no interaction between pesticide application (synthetic and botanical) and AMF on the degree of mycorrhizal infection, total population of soil bacteria, total population of soil fungi, plant phosphorus uptake, dry weight and maize crop yield. The application of synthetic and botanical pesticides significantly influences the reduction in the degree of mycorrhizal infection, the total population of soil bacteria, and increases the yield of maize plants. AMF inoculation significantly increased phosphorus uptake and maize yields.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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