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INCREASING SOIL SUPPRESSIVITY TO FUSARIUM WILT OF BANANA THROUGH BANANA INTERCROPPING WITH *Allium* spp.

PENINGKATAN SUPRESIFITAS TANAH TERHADAP PENYAKIT LAYU FUSARIUM PISANG MELALUI PERTANAMAN TUMPANGSARI ANTARA PISANG DAN Allium *spp*.

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ABSTRACT

Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *cubense* (Foc), is one of the most destructive diseases of banana and has spread in many plantation areas in Indonesia. Until today, the effective ways to control banana fusarium wilt disease have not yet been found. Some studies indicated that *Allium* spp. could be used to suppress plant diseases caused by *Fusarium*. *Allium* spp. are important horticultural crops which are generally cultivated in some areas in Indonesia. This research was conducted to determine the effect of several species of *Allium* spp. intercropped with banana to improve soil suppressiveness against banana fusarium wilt disease. The results showed that up to 12 months after planting, from 3 species of *Allium* spp. (*A. tuberosum*/Chinese leek, *A. fistulosum*/bunching onion, and *A. cepa* var. *aggregatum*/shallot) intercropped with banana Ambon Kuning (AAA) cultivar, Chinese leek and shallot were able to suppress the incidence of fusarium wilt disease of banana by 46 and 33% respectively. Soil analysis on the rhizosphere of banana intercropped with Chinese leek and shallot had lower population of total *Fusarium* compared to the other treatments. Analysis of fluorescein diacetate (3'.6'-diacetylfluoerescein) or FDA also showed that total microbial activity in the rhizosphere of banana intercropped with *Allium* spp. was also lower compared to control treatment (without intercopping). The observation of the effect of *Allium* spp. extracts on Foc showed that *Allium* spp. extracts were able to suppress the development of the colony and spore germination of Foc *in vitro*.

Keywords: *Allium* spp., banana, fusarium wilt suppression, intercropping

INTISARI

Layu Fusarium, yang disebabkan oleh Fusarium oxysporum *f.sp.* cubense *(Foc), merupakan salah satu penyakit tanaman pisang yang paling merusak dan telah tersebar di berbagai daerah di Indonesia. Sampai saat ini cara yang efektif untuk mengendalikan penyakit layu fusarium pisang belum ditemukan. Beberapa penelitian menunjukkan bahwa* Allium *spp. dapat dipergunakan untuk menekan penyakit tumbuhan yang disebabkan oleh* Fusarium*.* Allium *spp. adalah tanaman hortikultura penting yang banyak dibudidayakan di Indonesia. Penelitian ini dilakukan untuk mengetahui pengaruh beberapa spesies* Allium *spp. yang ditumpangsarikan dengan tanaman pisang untuk meningkatkan supresifitas tanah dalam menekan penyakit layu fusarium pisang. Hasil pengamatan menunjukkan bahwa sampai 12 bulan setelah tanam, dari 3 spesies* Allium *spp. (*A. tuberosum*/kucai,* A. fistulosum*/loncang, dan* A. cepa *var.* aggregatum*/ bawang merah) yang ditumpangsarikan dengan pisang kultivar Ambon Kuning (AAA), kucai dan bawang merah mampu menekan insidensi penyakit layu fusarium pisang berturut-turut sebesar 46% dan 33%.. Analisis tanah rhizosfer pisang yang ditumpang sari dengan kucai dan bawang merah menunjukkan populasi total Fusarium yang lebih rendah daripada perlakuan lainnya. Analisis* fluorescein diacetate (3'.6'-diacetylfluoerescein) *atau FDA juga menunjukkan bahwa aktivitas total mikrobia pada rhizosfer pisang yang ditumpang sari dengan* Allium *spp. lebih rendah jika dibandingkan dengan perlakuan kontrol (tanpa tumpang sari). Pengamatan pengaruh ekstrak* Allium *spp. terhadap Foc menunjukkan bahwa ekstrak* Allium *spp. mampu menekan perkembangan koloni dan perkecambahan spora Foc secara* in vitro*.*

Kata kunci: Allium *spp*., *penekanan layu fusarium, pisang, tumpang sari*

INTRODUCTION

Several constrains are faced to banana farmers in Indonesia, especially the attack of pest and plant pathogens. Fusarium wilt disease is the most damaging disease caused by *Fusarium oxysporum* f.sp. *cubense* (Foc) that attack banana plants through the root systems. According to Nasir *et al.* (2004) fusarium wilt caused the destruction of many commercial banana plantations in Indonesia, including in Riau, Jambi, Lampung, South Sulawesi, and Halmahera. Symptom of this disease is wilting which generally begins with yellowing leaves. The fungal pathogen spreads through soil, attacking the roots and entering banana rhizome. In the rhizome, the fungus grows, causes damage of vascular tissues, plant wilting and eventually death of the plant. Longitudinal splits may also develop in the pseudostem (Moore *et al*., 1995).

Intercropping is a system of planting several different crops in one place at the same time, as the main reason is to increase the productivity per unit area (Francis, 1986). When two or more species of plants grown at the same time, interaction will occur, each plant must have sufficient space to maximize cooperation and minimize competition. Therefore, various things are needeed to be consider in intercropping, such as: (1). Plants grown in intercropping should have different period of growth; (2). Plants have different needs for environmental factors such as air, moisture, light and nutrients; and (3). Plants have no allelopathy influences (Sullivan, 2003). The other purposes of intercropping system are to prevent pest attacks and to use more efficient land.

In this study, intercropping system were applied between banana and *Allium* spp. *Allium* spp. are suspected to produce several compounds which are able to inhibit the development of many fungal pathogens. It was expected that intercropping system between banana and *Allium* spp. can suppress the development of fusarium wilt disease caused by Foc. The activity of soil fungi and bacteria in this cropping system can be detected using Fluorescein diacetate (3'.6'-diacetylfluoerescein) or FDA analysis. Swisher and Carroll (1980) demonstrated that the amount of fluorescein produced by the hydrolysis of FDA was directly proportional to the microbial population growing on Douglas Fir foliage. The aims of this study are to determine the development of fusarium wilt disease of banana grown intercropped with *Allium* spp. and the effect of several extracts of *Allium* spp. on inhibiting the growth and sporulation of Foc *in vitro*.

MATERIALS AND METHODS

Effect of Ambon Kuning (AAA) Banana Cultivar Intercropped with Allium spp. to Fusarium Wilt Disease Development

The field experiment design was Randomized Complete Block Design with 5 different treatments of *Allium* spp. with 3 blocks as replications. The treatments were *A. cepa* var. *agregatum* (shallots), *A. tuberosum* (Chinese leek), *A. fistulosum* (bunching onion), and control. Ambon Kuning (AAA) banana cultivar and *Allium* spp. was planted on a plot with a size of 12.5×4 m^2. Each plot was planted with 8 of 2 months old banana seedlings obtained from tissue culture. *Allium* spp. were planted 4 times a year. Banana plants were planted at a spacing of 2×2.5 m^2 and at a spacing of 30×30 cm^2 for *Allium* spp. Banana plants were fertilized twice a year by using NPK with dose of 1 kg/plot, while *Allium* spp. by using ZA with dose of 1 kg/plot, SP36 of 0.5 kg/plot, KCl of 0.5 kg/plot, and urea fertilizer dose of 0.5 kg/plot. Fusarium wilt disease of banana observation was conducted every month by counting the incidence of the wilting plants.

Effect of Intercropping System on Total Population of Fusarium in Soil

The experimental design was Completely Randomized Design with 4 treatments of *Allium* spp. with 3 replications. Soil samples were collected at the end of experimental time. As much as 100 grams of soil samples were collected from around the rhizosphere of 8 banana plants, and were composited. Ten grams of soil samples were diluted in 90 mL of 0.02 M PBS solution (dilution 10^{-1}), and diluted again until to 10^{-3}. A total of 100 uL soil suspension was dropped onto PCNB medium and incubated for 7 days at room temperature. *Fusarium* formed white colonies on PCNB medium (Mandeel *et al*., 1995). Total population of *Fusarium* was calculated based on the number of white fungal colonies grown on the medium.

Total Microbial Activity

The total microbial activity was observed by measuring the fluorescein diacetate (FDA) hydrolytic activity in the soil. Five grams of air-dried soil was placed in an acetone resistant centrifuge tube, added with 20 ml of 60 mM sodium phosphate buffer pH 7.6 and 0.2 mL of 0.02% FDA solution, placed on mechanical shaker for 30 minutes. Reaction was stopped by adding 20 mL of acetone, and centrifuged for 10 minutes. A small amount of the supernatant was poured into spectrometer cuvette and the absorbance was read at a wavelength of 490 nm. Control should be performed with each soil samples to measure the

color not derived from the hydrolysis of FDA. The concentration of fluorescein released was calculated by reference to a standard curve of fluorescin (Adam & Duncan, 2001).

Effect of Allium spp. Extracts on Colony Growth of* Fusarium oxysporum *f. sp.* cubense *(Foc)

The experimental design was Completely Randomized Design with 4 treatments of *Allium* spp. extracts with 3 replications. Extracts of *Allium* spp. were obtained from 100 g of *Allium* spp. plants and 100 mL of aquadest, blended and filtered with filter paper. Extracts of *Allium* spp. plants were sterilized using 0.20 µm Millipore. One milliliter of *Allium* spp. extracts was added into 9 mL of warm liquid Potato Dextrose Agar, homogenized with a vortex, poured into a Petri dish filled with 2 drops of 25% lactic acid and allowed to stand until solid.

Effect of* Allium *spp. Extracts on Spore Germination of* Fusarium oxysporum *f. sp.* cubense *(Foc)

The experimental design was Completely Randomized Design with 4 treatments of *Allium* spp. extracts with 3 replications. One milliliter of *Allium* spp. extracts was added into 9 mL of warm liquid PDA, homogenized with a vortex, poured into a Petri dish filled with 2 drops of 25% lactic acid and allowed to stand until solid. Spores were harvested from 7 days old of pure culture of Foc BNT-2 isolate grown on PDA. Fifty microliter of spore suspension was dropped onto *Allium* spp. extract agar medium and incubated for 12 hours at room temperatur. Percentage of spore germination was observed for 100 microconidia.

Data Analysis

Data analysis was performed using ANOVA. If there is significantly different, it was followed by Duncan's Multiple Range Test at 95% significant level.

RESULTS AND DISCUSSION

Up to 12 months after planting, only shallot (*A. cepa* var. *aggregatum*) and Chinese leek (*A. tuberosum*) which were intercropped with Ambon Kuning (AAA) banana cultivar were able to suppress the incidence of banana fusarium wilt by 46% and 33% respectively, when compared to the control treatment (Figure 1). Study conducted in China showed that after 3 years Chinese leek cultivation, the incidence of fusarium wilt of banana decreased to less than 5% (Huang *et al*., 2012). This indicates that a field requires intensive cultivation of Chinese leek in a relatively long period of time to reduce *F. oxysporum* f.sp. *cubense* (Foc) attack before it can be used for growing bananas.

Low fusarium wilt disease incidence in Chinese leek and shallot treatments was probably related to the low Foc population in soil. Soil analysis conducted in this study showed that banana intercropped with Chinese leek (*A. tuberosum*) and shallot (*A. cepa* var. *aggregatum*) had the lowest total population of *Fusarium* spp. compared to other treatments (Figure 2). Presumably this was due to the existence of secondary metabolites released by particular *Allium* spp. that were toxic to *Fusarium* spp. into soil. Tagoe *et al*. (2011) mentioned that numerous naturally occurring phytochemicals are present in *Allium* plant tissue and many studies have evaluated their antimicrobial activities against fungi, bacteria, and viruses. Another study on stem rot of vanilla caused by *F. oxysporum* f.p. *vanillae* showed that rotation with *Allium* spp. and maize could reduce population of the fungal pathogen in soil due to the existance of antagonistic bacteria (Tombe, 2010).

Fluorescein diacetate (3'.6'-diacetylfluoerescein) or FDA has been used to determine the amount of active fungi and bacteria in soil. FDA is hydrolyzed by a number of different enzymes, such a proteases, lipases and esterases. The product of this enzymatic conversion is fluorescein. Fluorescein can be quantified by fluorometry or spectrophotometry (Schnurer & Rosswall, 1982). FDA has been used to estimate the potential microbial activity of soil amended with a wide range of organic matters and compared to the activity of soil microflora (Sanchez- Monedero *et al*., 2008). Although the secondary metabolites released by *Allium* spp. seemed to be able to suppress the population of Foc in soil, but they may also be toxic to other soil microorganisms as indicated by the fluorescein diacetate (FDA) analysis (Figure 3). In this study the total microbial activity in soil planted with banana intercropped with *Allium* spp. showed lower amount of FDA hydrolysis activity compared to control treatment except for bunching onion (*A. fistulosum*) (Figure 3). It means that bunching onion (*A. fistulosum*) had a little effect for reducing soil microorganism population including soil pathogens such as Foc.

The low Foc population in soil might be related to the inhibition of the growth of Foc. This study showed that after 7 days of incubation in room temperature, the colony diameter of Foc grown on most *Allium* spp. extracts media, such as *A. cepa* var. *aggregatum* (shallot), *A. tuberosum* (Chinese leek), and *A. fistulosum* (bunching onion) was suppressed (Figure 4 and 5). Germination percentage of spores taken from Foc grown on *Allium* spp. extract medium was as high as control treatment (data not shown). This suggests that the inhibitory effect of the extract *Allium* spp. on Foc growth is fungistatic and not merely be fungicidal.

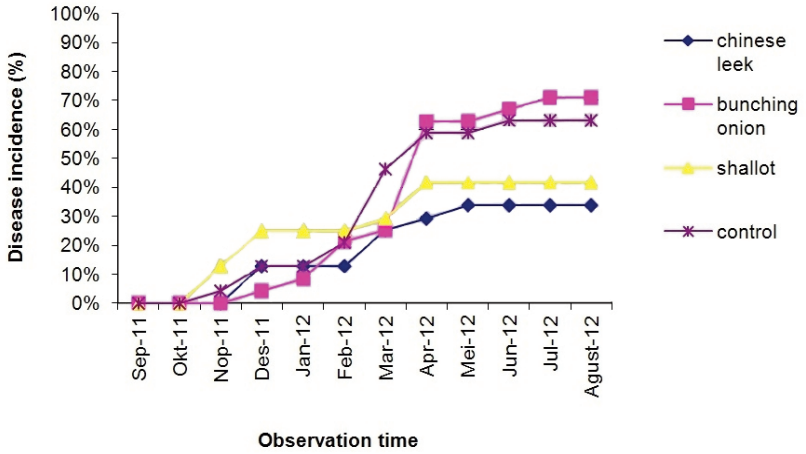

Figure 1. Development of fusarium wilt disease of Ambon Kuning (AAA) banana cultivar intercropped with *Allium* spp.

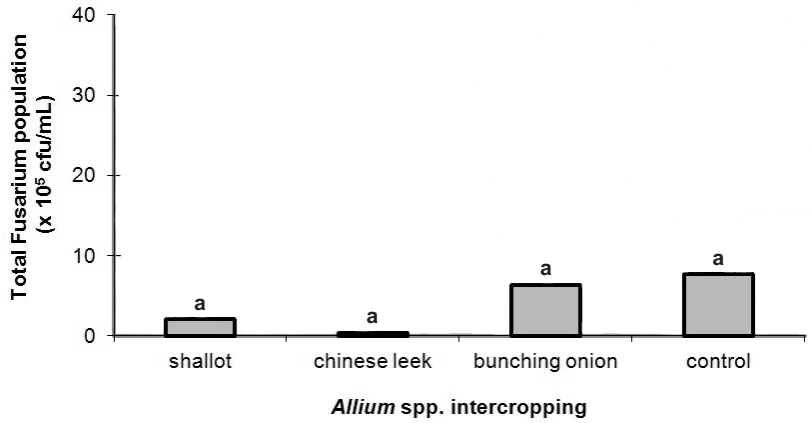

Figure 2. Total *Fusarium* population in soil planted with Ambon Kuning (AAA) banana cultivar intercropped with *Allium* spp.

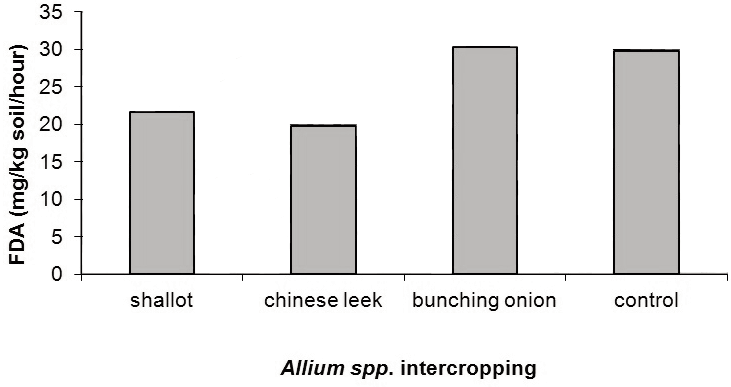

Figure 3. Total microbial activity in soil planted with Ambon Kuning (AAA) banana cultivar intercropped with different *Allium* spp.

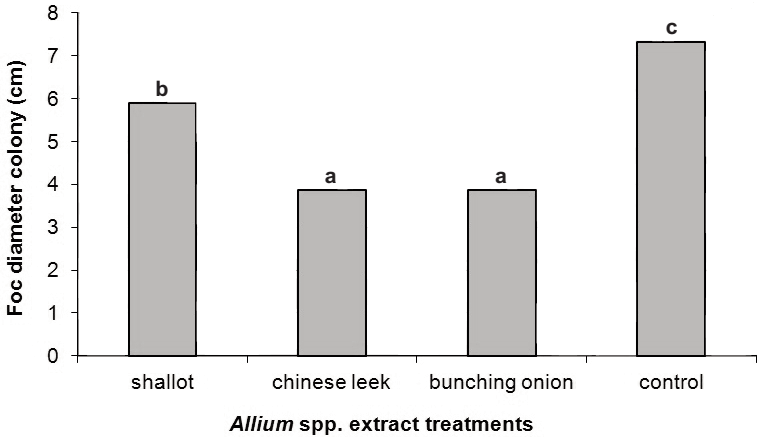

Figure 4. Diameter colony of *F. oxysporum* f.sp. *cubense* on PDA amended with different *Allium* spp. extracts after 7 days of incubation

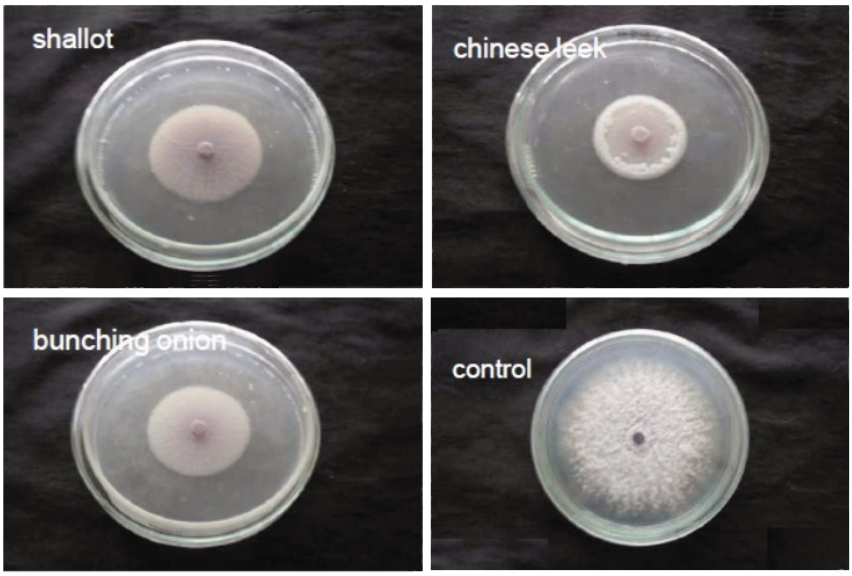

Figure 5. The suppression of *F. oxysporum* f.sp. *cubense* colony development on PDA amended with different extracts of *Allium* spp. after 7 days of incubation

Some studies showed that *Allium* spp. extracts such as *A. sativum*, *A. ursinum* and *A. obliqum* were able to inhibit the development of *Fusarium oxysporum* (Abd El-Ghany *et al*., 2015; Parvu & Parvu, 2011). Zhang *et al*. (2012) showed that aqueous leachates of both roots and leaves of chinese leek inhibited colony growth of Foc. Root leachates were more potent to inhibit Foc than leaf leachates. Zhang *et al*. (2012) also showed that the volatile released from both leaves and roots of chinese leek inhibited the mycelial growth of Foc.

Parvu *et al*. (2010) showed that when *Fusarium oxysporum* f.sp. *tulipae* hyphae was treated with *A. fistulosum* plant extract, there would be ultrastructural changes, i.e., the external sheath was slightly modified and the cell wall had irregular shape on the outside, the organelles were partly or entirely destroyed, the cytoplasm was degenerated and electron dense material appeared in the hyphal cells. The precipitation of the cytoplasm and the destruction of the organelles and nucleus caused the loss of hyphae's viability.

The toxicity of *Allium* spp. extracts was also showed by the inhibition of fungal spore germination. The percentage of spore germination of Foc cultured on PDA amended with different *Allium* spp. (*A. cepa* var. *aggregatum*/shallot, A. *tuberosum*/ Chinese leek, and *A. fistulosum*/ bunching onion) extracts was suppressed compared to control treatment (Figure 6 and 7). This finding was consistent with the result obtained by Huang *et al*. (2012) which showed that the crude extract of Chinese leek (*A. tuberosum*) resulted in mortality of Foc race 4 spores up to 87%.

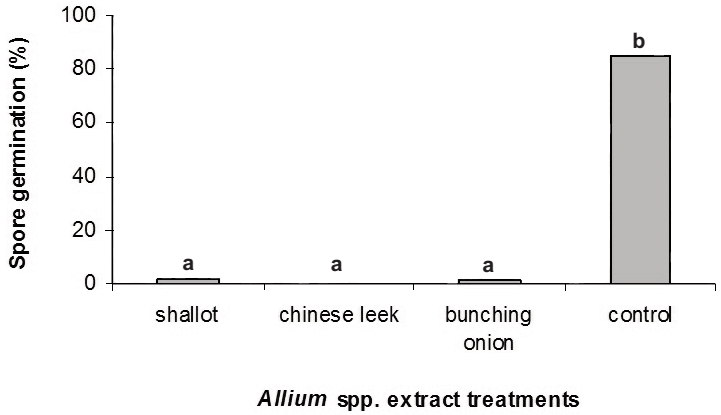

Figure 6. Percentage of spore germination of *F. oxysporum* f.sp. *cubense* cultured on PDA amended with different *Allium* spp. extract

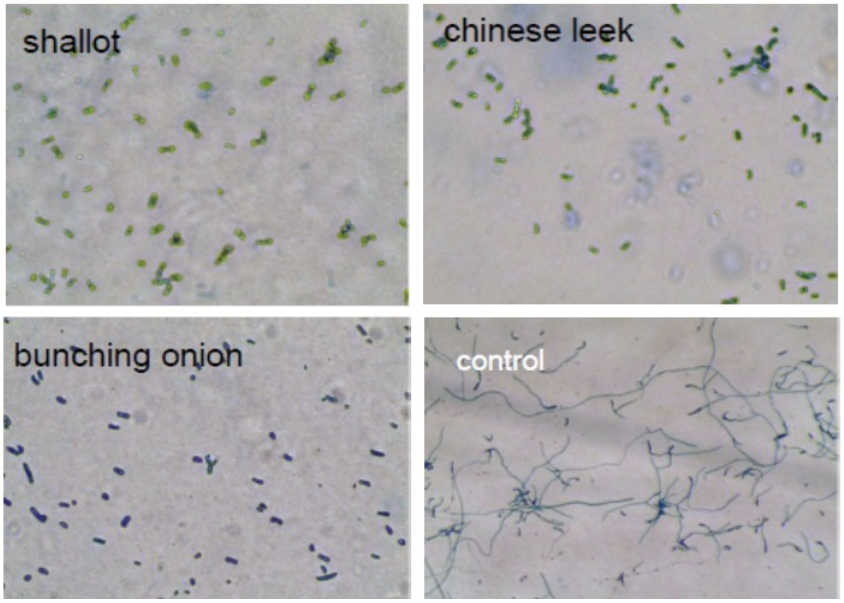

Figure 7. Spore germination of *Fusarium oxysporum* f.sp. *cubense* on PDA medium amended with different *Allium* spp. extracts

Some studies showed that *Allium* plant extracts was able to inhibit spore germination of some plant pathogenic fungi. Un-Nisa *et al*. (2010) showed that plant extracts of *A. sativum* and *A. cepa* could suppress the spore germination of *Alternaria alternata* and *Rhizopus stolonifer*, and also *Fusarium oxysporum* (Un-Nisa *et al*., 2011). *Allium* plant extracts contain different chemical compounds. In *A. cepa*, *A. sativum*, and *A. ampeloprasum* extracts, different biologically active substance, such as organosulphures compounds like alliin and allicin, sterols, flavones, and and polyphenolcarboxylic acid have been found. Alliin is the precursor of allicin, formed by the action of allinase enzyme. Allicin has antibacterial and antiviral effects. It is also efficient against many fungal species, such as *Aspergillus flavus*, *A. niger*, *Candida albicans*, *Fusarium laceratum*, *Microsporum canis*, *Mucor racemosus*, *Penicillium* spp., *Rhizopus nigricans*, *Saccharomyces* spp., *Trichophyton granulosum*, *F. oxysporum*, *B. cinerea*, *B. paeoniae*, *P. gladioli*, and *S. sclerotiorum* (Parvu & Parvu, 2011).

CONCLUSION

Intercropping between Ambon kuning (AAA) banana cultivar with *Allium cepa* var. *aggregatum* (shallot) or *A. tuberosum* (Chinese leek) could suppress the development of fusarium wilt of banana. Plant extracts of *Allium cepa* var. *aggregatum* (shallot), *A. tuberosum* (Chinese leek) and *A. fistulosum* (bunching onion) could suppress Foc growth and spore germination *in vitro*.

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