DECREASE CLUBROOT DISEASE INTENSITY OF *Brassica juncea* ON Pb CONTAMINATED SOIL USING *Paraserianthes falcataria* THAT WAS INFECTED BY Mycorrhizal

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ABSTRACT

Both, biotic and abiotic factor is the most important problem for agriculture in Indonesia. Biotic factor such as plant disease could decrease yield till more than 50%, while abiotic factor such as soil contamination could decrease yield till more than 30%. One of choice to overcome this problem is using bio-phytoremediation method. This method combine remediation using plant (phytoremediation) and remediation using microbe (bio remediation). This study was aimed to determine the effects of bio-phytotemediation method using *Paraserianthes falcataria* which infected by mycorrhizal fungi to overcome *Phytophthora brassicae*, the main clubroot disease on *Brassica juncea*, in Pb contaminated soil. We also aimed to know the influence of the method to absorb soil Pb using *Paraserianthes falcataria*. The research was conducted in the Mycology Faculty of Agriculture, University of Brawijaya from March to October 2015. The research used Completely Randomized Design with 6 treatments and 4 replications. Parameter observation in this research were: 1) Intensity of clubroot disease; 2) Growth of *B. juncea*; 3) Population of mycorrhizal spores in soil, 4) Mycorrhizal infection inside plant cell; and 4) Pb content of soil and plant tissues. The results was showed that mycorrhizal has significantly effects to decrease clubroot disease intensity. But, mycorrhizal has no significantly effect to the plant growth, including stem length and leaves number. The number of mycorrhizal spores in the soil after 35 days application was increases, and the percentage of infection in the roots of *B. juncea* and *P. falcataria* was fluctuated. The application of mycorrhizal can decrease Pb content in the soil and increase Pb content in *P. falcataria*.

Keywords: *Abiotic factors, biotic factors, mycorrhizal, Pb, phytoremediation, Plasmodiophora brassicae.*

1. INTRODUCTION

One of the most common agricultural commodities grown in Indonesia is *B. juncea*. It's production influenced by several factors such as plant disease and environments. Sastrahidayat (2011) stated that the diseases of plants can be caused by biotic and abiotic factors.

Pathogens *P. brassicae* is one of the biotic factor that cause clubroot disease in *B. juncea*. While one of the abiotic factors that cause disease in plants is the pollution of Pb (lead).

P. falcataria is one of the plants that can be used to reduce the stress of Pb in soils (Rossiana, 2007). Absorption of Pb metal in *P. falcataria* can be increased by inoculation of mycorrhizal. Infection of mycorrhizal inside plant's cell can protect it from diseases such as clubroot (Husna, *et al*., 2007), and also can increase absorption of metal in plants (Aprilia and Purwani, 2013).

This study was aimed to determine the effects of bio-phytotemediation application using *Paraserianthes falcataria* which infected by mycorrhizal fungi to overcome *P. brassicae*, the clubroot disease of *Brassica juncea*, in Pb contaminated soil. We also aimed to know the influence of the method to absorb soil Pb.

2. METHODS

The research was conducted in the Mycology Laboratory of Agriculture Faculty,

Brawijaya University. Research has been started from March till October 2015. The research design was using Completely Randomized Design with 6 treatments and 4 replications. The independent factors were mycorrhizal doses. The doses were: P0 = 0 g, P1 = 20 g, P2 = 40 g, P3 = 60 g, P4 = 80 g, P5 = 100 g.

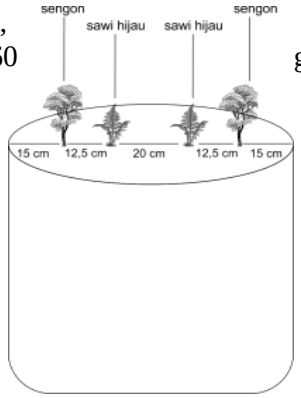

Figure 1. Position of *P. falcataria* (sengon) and *B. juncea* (sawi hijau) on trashbag.

Sterilization of media

Sterilization was done by spraying formalin at 5% concentration at dose 25 ml per kg soil. Soil then was covered for 7 days, then after 7 days later it was dried. The sterile soil then contaminated with 100 ppm of Pb metals.

Pb solution was made by mix 4g of $Pb(NO^3)^2$ with 15 ml of HNO^3 in 1000 ml measuring cup, then diluted with distilled water till reach 1000 ml. This solution is equivalent to 4000 mg per liter or 4000 ppm. Then Pb solution was poured into 40 kg of sterile soil.

Soil that has been contaminated with Pb then inoculated with *P. brassicae*. Suspension of *P. brassicae* was made by cut the roots of *B. juncea* that infected by clubroots disease into small pieces. Suspension of *P. brassicae* then poured in the soil at dose of 250 ml per trashbag.

Inoculation of mychorrizal fungi

Spore of mycorrhizal fungi was inoculated in rhyzosphere zone in trashbag. *B. juncea* was grown 15 days after *P. falcataria* was grown. Plant cultivation including watering and weeding.

Parameter observation including the intensity of clubroot disease, the growth of *B. juncea*, Pb content in soil and plant tissues, and mycorrhizal spore population.

The intensity of disease was calculated by scoring value using the formula (Bjorling, 2013) that has been modified;

$$IP = \frac{\sum(n\ x\ 0+n\ x\ 1+n\ x\ 2+n\ x\ 3+n\ x\ 4)}{\sum plants}\ x\ 100\%$$

Where IP = Intensity Disease; n = number of plants with scale attacks; 0-4 = scale attack

Table 1. Symptom Scale

Scale	Root symptom
0	There are no symptoms of clubroot
1	1-25% root damage
2	26-50% root damage
3	51-75% root damage
4	More than 75% root damage

B. juncea vegetative growth including stem length and leaves number. Stem length was calculated from the ground to the base of the youngest leaf.

Analysis of Pb on soil and plant tissue using spectrophotometer type Shimadzu UV 1800.

Observations of mychorrizal include the number of mycorrhizal spores and infection of plant roots.

Number of spores was observed using sieving and decanting method. Soil was put into the sieve-storey (200 µm, 150 µm, and 45 µm). Soil retained on 45 µm sieve was poured into a beakerglass. The solution was put in the tub and added with 60% sugar and then centrifuged at 3000 rpm for 3 minutes. While the observation of mycorrhizal infection inside plant cell was done by cut plant roots along the ± 1 cm pieces. Root pieces was boiled in 10% of KOH solution for 20 minutes, then was rinsed with tap water for 4 times. Next step was soaked the root in a solution of H_2O_2 for 10 minutes, and then was soaked with 1% of HCL for 10 minutes. Furthermore, the root then was boiled in 1% of lactophenol tryphan blue (LTB) for 10 minutes. The roots was arranged on object glass to be observed microscopically.

Mycorrhizal infection percentage was calculated using formula:

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Infection Percentage: $\frac{Infected\ roots}{Observed\ roots}\ x\ 100\%$

Data Analysis

The data of intensity clubroot disease percentage (%), stem length (cm), and number of leaves of *B. juncea* were analyzed by analysis of variance (ANOVA) and the significantly data will be continued analyzed with Duncan Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

Clubroot Diseases in B. juncea

B. juncea which attacked by *P. brassicae* pathogen was showed swelling symptoms in the roots area. In severe attacks would showed root decay. Microscopically observations was showed that the spores of *P. brassicae* has round and colorless transparent.

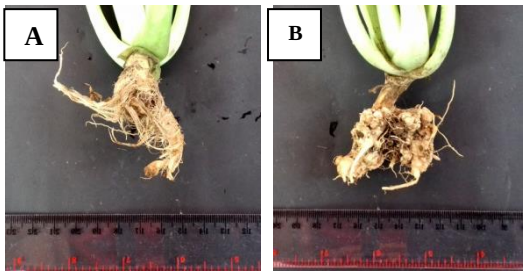

Figure 2. (A) Health root; (B) Infected root

Clubroot Disease Intensity

ANOVA for clubroot disease intensity in *B. juncea* showed that mycorrhizal treatment has significantly effect on clubroot disease intensity.

Clubroot disease intensity on *B. juncea* was declined and it's was expected because of mycorrhizal hyphae presence that surround plant's roots as a direct barrier of pathogen penetration. In addition, mycorrhizal that infect plant roots and forms arbuscular and vesicles inside plant's cell and fullfill the root cells can suppress the development of pathogens inside root's cells.

Table 2. The intensity of clubroot disease

Treatments	Clubroot disease intensity (%)
P0	66 b
P1	53 ab
P2	47 ab
P3	44 ab
P4	28 a
P5	25 a

Where:
- The figures accompanied by the same letters in the same column shows not significantly different based on test Duncan (DMRT) at the level of 95%.
- Data transformed Arc Sin for analysis.

Plant's roots that colonized or infected by beneficial microorganisms like mycorrhizal in the soil can reduce contact between plants and pathogen (Cicu, 2005). Development of *P. brassicae* pathogen in the roots can be hampered by arbuscular inside plant root cells. (Hajoeningtijas and Budi, 2005).

Vegetative Growth

Vegetative growth of *B. juncea* include stem length and number of leaves is showed on Table 3.

Table 3. *S*tem length and leaves number of *B. juncea*

Treatments	The average stem length (cm)	The average number of leaves
P0	3,68 a	8,00 a
P1	5,24 b	8,50 a
P2	4,85 b	8,38 a
P3	3,55 a	8,38 a
P4	5,41 b	8,75 a
P5	3,60 a	8,00 a

Where:
- The figures accompanied by the same letters in the same column shows not significantly different based on test Duncan (DMRT) at the level of 95%.

Results of analysis of variance stem length and number of leaves of *B. juncea* showed dose of 0-100 g mycorrhizal not significantly affect to the stem length and leaves of *B. juncea*. This is caused because of space, light, and nutrients competition between *B. juncea* and *P. falcataria*.

P. falcataria which was grown intercrop with *B. juncea* reduced the space for *B. juncea* growth. Otherwise, the existence of the *P. falcataria* also compete for nutrients and light. Canopy wide and plant height of *P. falcataria* that wider and taller than *B. juncea* reduced light intensity. Consequently it would disrupt photosynthesis in the leaves of *B. juncea.*

According to Kadir (2014), that plant spacing and fertilizer, both alone or together did not significantly affect to plant height and leaves number of *B. juncea*.

Other factors that influence the growth of *B. juncea* is Pb metal and *P. brassicae* pathogen. Pb that contamined soil at 90 mg/kg (90 ppm) would cause necrosis, chlorosis, and die (Mangkoedihardjo *et al*., 2005). *P. brassicae's* root that was infected by pathogen would be enlarged so the roots unable to absorb water and nutrients and the plants will be stunted (Sastrahidayat, 2011).

Number of Mycorrhizal Spores and Percentages of Mycorrhizal Infection

Number of mycorrhizal spores in the soil after 35 day after planting showed increase. Control treatments showed 9 spores, this is because mycorrhizal already exist in the soil naturally. According to Husna *et al*. (2007) arbuscular mycorrhizal is a potential biological resources found in nature and almost can be found in a variety of ecosystem.

Table 4. Number of mycorrhizal spores in the soil after 35 day after planting

Treatments	Number of spore/10 g soil
P0	9,00 a
P1	22,00 b
P2	42 c
P3	87 d
P4	119 e
P5	151 f

Mycorrhizal symbiosis with the plant can be determined by observe root infection. A symbiosis occurs when mycorrhizal fungi infect plant roots. Mycorrhizal infection on the roots of plants characterized by vesicles (mycorrhizal structures are round or oval which serves as a storage of food reserves) or arbuscular (mycorrhizal structures shaped like a tree that serves as a food exchange between mycorrhizal with its host).

The higher dose of mycorrhizal not always increase mycorrhizal infection. Different species of mycorrhizal has different abilities to infect plant roots.

Table 5. Percentage of infection mycorrhizal

Treatment	Percentage of infection (25 root samples)	
	P. falcataria (%)	*B. juncea* (%)
P0	0	0
P1	40	44
P2	28	40
P3	28	44
P4	48	52
P5	60	52

According to Solaiman and Hirata (1995) in Nurhayati (2012), that mycorrhizal infectivity is influenced by several things: the species, host plant, interaction of microbial, root type of host plants, and competition between mycorrhizal fungi that called biotic factors, and environmental that called abiotic factors.

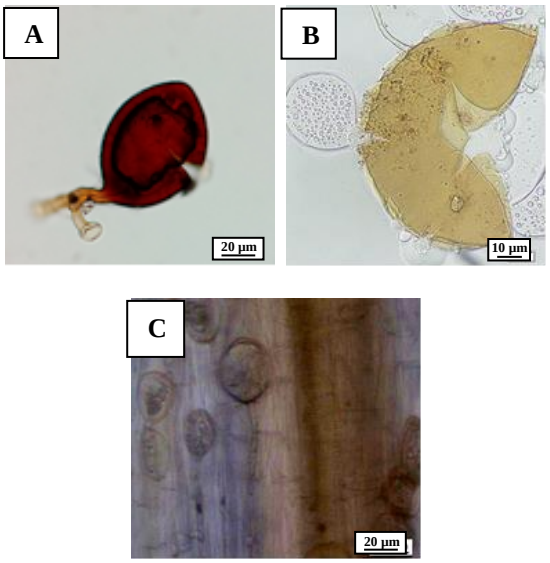

Figure 3. Mycorrhizal. (A) Spore; (B) Broken spore; (C) Infection of plant root

Analysis of Pb Content in Soil and Plant

Declining of Pb content in soil showed absorption of Pb by plants while phytoremediation occurs. Pb metal decrease in in the soil along with increasing mycorrhizal dose indicates application of mycorrhizal can increasing Pb absorption by plants.

Table 6. Analysis Pb metal in the soil

Treatments	Analysis Pb metal in the soil (mg/kg)		
	Before contamination	After contamination	After 35 day after planting
P0	4,213	27,841	27,532
P1	4,213	27,841	27,391
P2	4,213	27,841	27,189

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P3	4,213	27,841	27,079
P4	4,213	27,841	26,896
P5	4,213	27,841	26,617

The content of Pb metal in soil decreases with increasing of application mycorrhizal. The benefits of mycorrhizal in helping the absorption of Pb in plants is store the metal in its hyphae. Beside in the soil, analysis of Pb metal content is also carried out in the plant tissue of *P. falcataria* and *B. juncea* at age 35 day after planting. Plant tissue used in the analysis are the roots, stems, and leaves.

The results of the analysis of absorption of Pb in the plant tissue *P. falcataria* and *B. juncea* showed that more increased mycorrhizal dose causes increased absorption of Pb by plants.

Mycorrhizal symbiosis with the plant cause wide area coverage to absorp nutrients and water including Pb is extend. Increased Pb that can be absorbed cause the advance of Pb metal content in plants. In addition, high uptake of Pb in plants caused Pb absorbed and stored by mycorrhizal in their braid hyphae, so that the plants with mycorrhizal can absorb metal higher.

According to Husna *et al*. (2007) the use of mycorrhizal has several advantages, such as helping plants to absorb macro and micro nutrients, more get water because it can reach micro porous soil that is not reachable by the hair roots, increased resistance to drought, increased resistance against root pathogens (improvement of plant nutrients, the hyphae layer covering the roots, releasing antibiotics), heavy metal pollution (the mechanism of fungal hyphae) and salinity, mycorrhizal can also produce hormones that can stimulate plant growth.

Table 7. Analysis absorption Pb metal in plant tissue of *P. falcataria* and *B. juncea*

Treatments	*P. falcataria* (mg/kg)				*B. juncea* (mg/kg)			
	Root	Stem	Leaves	Amount of Pb absorption	Root	Stem	Leaves	Amount of Pb absorption
P0	0,0894	0,0573	0,0278	0,1745	0,0288	0,0288	0,0148	0,0724
P1	0,1332	0,0860	0,0427	0,2620	0,0420	0,0436	0,0215	0,1071
P2	0,1863	0,1128	0,0585	0,3576	0,0606	0,0614	0,0304	0,1523
P3	0,2289	0,1427	0,0747	0,4463	0,0740	0,0751	0,0372	0,1862
P4	0,2802	0,1825	0,0921	0,5548	0,0931	0,0922	0,0465	0,2318
P5	0,3622	0,2279	0,1207	0,7108	0,1117	0,1111	0,0592	0,2820

4. CONCLUSION

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

1. The application of mycorrhizal has significant effect to decrease the intensity of clubroot disease caused by *P. brassicae* on *B. juncea* in Pb contaminated land.
2. Increasing of mycorrhizae dose from 0 to 100 gram can effects the increasing of Pb metal by *P. falcataria* and reducing the Pb metal content in the soil.

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