PRETREATMENT EFFECT OF BLACK PEPPER SEEDLINGS WITH *Pseudomonas, Trichoderma* AND MYCORRHIZA ON FOOT ROT DISEASE INCIDENCE

Pengaruh perlakuan Pseudomonas, Trichoderma *dan Mikoriza Arbuskula pada benih lada terhadap kejadian Busuk Pangkal Batang*

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(diterima 27 April 2015, direvisi 29 Juli 2015, disetujui 23 Februari 2016)

ABSTRACT

Foot rot disease caused by a *Phytophthora capsici* is a main constraint of black pepper cultivation in Indonesia. Improving soil microbial community populations are an alternative approach to suppress the disease incidence. The objective of the present study was assessing the soil microbe application on disease incidence. The research was conducted at greenhouse and field site in Bogor and Sukamulya Research Station, Sukabumi, West Java, Indonesia. At the greenhouse: Seedlings of Natar 1 variety derived from a single node cutting were inoculated with: (1) *P. fluorescens* (Pf); (2) *Trichoderma* (Tr); (3) *P. fluorescens* + *Trichoderma* (Pf+Tr); (4) *P. fluorescens* + *Trichoderma* + AM fungus (Pf+Tr+AM); that artificially inoculated with *Phytophthor*a; (5) The untreated treatment (0) and (6) the *P. capsici* inoculated (Phy) were used as control. The experiment was arranged in Complete Random Design, repeated three times, with 30 seedlings each. The field trial was performed at foot rot disease endemic site. The seven nodes pepper seedlings that previously inoculated with (1) *P. fluorescens* (Pf), (2) *Trichoderma* (Tr), (3) *P. fluorescens* and *Trichoderma* (Pf+Tr), (4) *P. fluorescens, Trichoderma,* and AM fungi (Pf+Tr+AM), and the uninoculated (0) were used as materials. The treatments were arranged in Complete Random Block Design with five plots each, which consist of 16 pepper vines each. Observations were carried out for disease incidence, soil microbes population and plant vegetative growth parameters. The results showed, all tested beneficial soil microbes reduced disease incidence occurrence for greenhouse, however only *Trichoderma* individual treatment reduced disease incidence lower than the control in the field test.

Key words: Arbuscular Mycorrhiza, Black pepper, *Pseudomonas fluorescens, Phytophthora capsici, Trichoderma*

ABSTRAK

Penyakit Busuk Pangkal Batang (BPB) lada yang disebabkan cendawan Phytophthora capsici *masih menjadi kendala dalam budidaya lada di Indonesia. Memperbaiki populasi mikroba bermanfaat di dalam tanah merupakan alternatif pengendalian penyakit BPB. Penelitian bertujuan mengetahui efektifitas aplikasi mikroba dalam menekan kejadian BPB. Penelitian dilakukan di rumah kaca Balittro di Bogor dan dilanjutkan di lapangan, di KP Sukamulya, Sukabumi, Indonesia. Setek satu buku varietas lada Natar 1, diaplikasi dengan enam perlakuan, yaitu inokulasi (1)* P. fluorescens *(Pf); (2)* Trichoderma *(Tr); (3)* P. fluorescens + Trichoderma *(Pf+Tr); (4)* P. fluorescens + Trichoderma *+ mikoriza arbuskula (Pf+Tr+AM). Semua perlakuan tersebut dikombinasikan dengan inokulasi* P. capsici *(Phy); (5) Bibit yang tidak diinokulasi (0), dan (6) diinokulasi* Phytophthora *saja (Phy) sebagai kontrol. Percobaan disusun dalam rancangan acak lengkap, diulang tiga kali terdiri atas 30 bibit tiap ulangan. Percobaan di lapang dilaksanakan di daerah endemik Phytophthora. Perlakuan yang diuji adalah bibit lada yang sebelumnya telah diinokulasi dengan (1)* P. fluorescens *(Pf), (2)* Trichoderma *(Tr), (3)* P. fluorescens + Trichoderma *(Pf+Tr), (4)* P. fluorescens + Trichoderma+ *mikoriza arbuskula mikoriza (Pf+Tr+AM), serta (5) Kontrol/tanpa perlakuan inokulasi (0). Percobaan disusun dalam RAK, dengan lima blok ulangan, setiap blok terdiri atas16 bibit lada. Pengamatan dilakukan terhadap kejadian busuk pangkal batang, populasi mikroba di dalam tanah, pertumbuhan vegetatif tanaman. Hasil di rumah kaca, semua* *mikroba bermanfaat yang diaplikasikan menekan kejadian penyakit, tetapi di lapang hanya perlakuan* Trichoderma *yang dapat menurunkan kejadian penyakit BPB lebih rendah dibanding kontrol.*

***Kata kunci**: Mikoriza arbuskula,* Piper nigrum*,* Pseudomonas fluorescens*,* Phytophthora capsici, Trichoderma

INTRODUCTION

Black pepper (*Piper nigrum* L) contributed the seventh biggest earn of exporting commodity among the existing estate crops in Indonesia. Lampung province with its brand market Lampung Black Pepper, and Bangka-Belitung province with Muntok White Pepper are well known as black pepper producing areas (Manohara *et al.,* 2005).

Foot rot disease caused by a soil borne plant parasitic fungus *Phytophthora capsici* is a serious constraint in black pepper cultivation in Indonesia. Contaminated planting materials or infected black pepper vine seedlings are important means in disease distribution (Manohara *et al.,* 2005, Anandaraj, 2005). The disease has been found in several important pepper producing areas of Indonesia (Wahyuno *et al.,* 2010). Two mating types (A1 and A2) of *P. capsici* population were also found in Lampung, Kalimantan and others cultivation area of black pepper (Manohara and Sato, 1992); those are vary in their virulence against *Piper* spp. (Wahyuno *et al.,* 2010). The disease caused yield lost of about 19.6 billion rupiah annually (Ditlintanbun, 2007).

The resistant variety of black pepper to *P. capsici* has not been available yet. An attempt to minimize foot rot disease, mostly emphasized by improving cultural practices and also developing a standard protocol for black pepper mass propagation (Manohara *et al.,* 2005). Application of *Trichoderma* had also been tested in the green house and an infected farmer's garden (Wahyuno *et al.,* 2003; 2007). However, the disease still exists because the pathogen is also distributed through planting materials, and the available technology cultivation *e.g*. Good Agricultural Practices on black pepper were not applied by the farmers, as well. Therefore, ensuring planting materials such as pepper vine seedlings-free of the pathogen is crucial in order to minimize fungal spreading and yield losses.

An application of beneficial soil microbes such as *Trichoderma* and *Pseudomonas fluorescens* individually or in combination starting from black pepper vines seedling preparation is considered to give an advantage in plant protection and, perhaps also the plant growth. Pearson and Weller (1994) assumed that the mixture strain of *Pseudomonas fluorescens* may create more stable rhizosphere community and more diverse spectrum of biocontrol mechanisms and possibly more consistent in disease suppression. Drenched *P. fluorescens* individually into the soil medium before black pepper planting reduced wilt disease caused by *P. capsici* at the greenhouse level (Anith, 2002). Application of *Trichoderma* and *P. fluorescens* individually enhance microelement absorption, increase root length and dry weight, widening leaf area, and also plant height on soybean seedlings (Entesari *et al.,* 2013); induce resistance against *Fusarium* on cucumber, and against *Botrytis cinera* in *Arabidopsis* when both of microbes were applied in combination (Alizadeh *et al.,* 2013). Consortium approach of compatible-mixture of *Trichoderma* and bacterial strains need to be developed in order to provide better protection of black pepper from soil borne pathogens (Sharma and Kallo, 2004). Application of a mixed of *Trichoderma* and Arbuscular Mycorrhiza (AM) fungi promoted growth of chili pepper (Bhuvaneswari *et al.,* 2014). Effectiveness of *P. fluorescens* against *Fusarium* on watermelon depends on the strain of bacteria and combinations of the two bacteria effectively reduced the disease up to the first three weeks after application (Tziros *et al.,* 2007). Inoculation of Arbuscular Mycorrhiza, and *Glomus* spp enhance growth of bushy pepper (Trisilawati and Rochmat, 2005). This informations indicate that the effect of beneficial microbes' application are varied according to many factors. Understanding biological characteristic of suppressive soil is a prerequisite to suppressive soil approaches in soil borne disease control (Mazzola, 2004).

The objective of the present study was evaluating the effectiveness of preinoculation of black pepper seedlings with *P. fluorescens*, *Trichoderma,* and Arbuscular Mycorrhiza (AM) on foot rot disease incidence of black pepper seedling in the green house and field.

MATERIAL AND METHOD

The experiments were conducted in the green house and Sukamulya Research Station of Indonesian Spice and Medicinal Crops Research Institute (ISMCRI), in Bogor and Sukabumi, West Java, respectively, from January 2012 until December 2014. The *P. fluorescens* isolates were screened by growing the bacteria in King's B Medium for emitting fluorescens characteristic and sub cultured onto Nutrient Agar (NA) medium for subsequent preservation prior to dual culture test against *Phytophthora capsici*. A *Pseudomonas fluorescens* (cm-8) and *Trichoderma harzianum* (Skm) isolates were selected after both of microbes showed higher inhibition zone during the dual culture test against *P. capsici* (K2) in *in vitro* test on Potato Dextrose Agar (PDA) medium. The PDA medium was used for preservation of selected *Trichoderma* instead of NA medium. The AM fungus inoculums, *Glomus* spp (Mycho-PN) was obtained from infected root of black pepper taken from Sukabumi, West Java, which were multiplied by growing the spore inoculums on the root of *Sorghum* and preserved in mix fern-zeolite medium prior to inoculation on to pepper seedlings of respective treatment. The *P. fluorescens*, *Trichoderma, Phytophthora* and AM fungus used in the present study were belongs to ISMCRI culture collections.

These serial experiments consist of, a) testing the effect of beneficial microorganisms application on pepper vines in green house (at 24-30^0 C), and b) evaluation of the inoculated black pepper vines by using beneficial microorganisms in the field trial at the foot rot disease endemic area.

Green house test

The medium used was steam-pasteurized soil consist of soil + cow-dung manure (2:1) in polybag (± 2 kg weight). The treatments tested were 1) *Pseudomonas fluorescens* (Pf)*;* 2) *Trichoderma* (Tr)*;* 3) *P. fluorescens + Trichoderma* (Pf + Tr); 4) *P. fluorescens + Trichoderma* + AM fungus (Pf + Tr + AM); 5) Untreated control (0); and 6) inoculated control with *P. capsici* (Phy).

The suspension of *P. fluorescens* (Cm-8) and conidial suspension of *Trichoderma* (T-48) were inoculated into soil medium with 0.1% molasses as carrier and food base, by using a modified method of Beary *et al.* (2002). The modification was lowering the molasses application (0.1%), instead of 0.3%. The population density for *P. fluorescens* was ± 10^8 cfu ml^{-1} and ± 10^7 conidia ml^{-1} for *Trichoderma* diluting in 0.1% molasses and drenched as many as 50 ml $polybag^{-1}$ at respective treatments, then incubated for two weeks before used as planting medium. No additional suspension of *P. fluorescens* or *Trichoderma* were drenched for the control treatment. Single node cutting peppers were prepared by planting the vines of Natar 1 variety onto those prepared planting medium. For the AM fungus treatment, additional inoculation of about 10 g mixture of spores of *Glomus* spp in fern-zeolite carrier (26 spores g^{-1} fern-zeolit carrier) was spread along the pepper vine areas. The black pepper vine-cuttings were maintained in plastic house for four months at shading area till three to four new nodes formed and the vines were adapted to the open air. The zoospore suspension of *P. capsici* was inoculated on to respective treatments by drenching of 10^5 zoospores ml^{-1} as many as 50 ml for each polybag, around the vine rhizosphere

(Wahyuno *et al.,* 2009), and foot rot disease incidence as indicated by the quick wilt symptom was monitored monthly. All the treatments were repeated three times, which consist of 30 polybags each and arranged in complete randomized design.

Disease incidence was measured three months after the application of *Phytophthora* suspension followed the formula of Johnston and Booth (1983). The total bacteria population density in the soil planting medium was counted at the end of observation by perfoming dilution plate method on NA medium. Amended the PDA medium with 250 $mg.l^{-1}$ of chloramphenicol was used for counting the total fungi. *Trichoderma* selective medium that consist of chloramphenicol (250 $mg.l^{-1}$) and rose bengal (150 $mg.l^{-1}$) were used for counting *Trichoderma* population based on Dhingra and Sinclair (1995). Phosphorous and salicylic acid content in the roots was analyzed at seventh month after planting. When the black pepper vine seedlings had arisen of about seven nodes, then the seedlings were replanted in the field. The roots were collected by digging the soil medium, washing the root and preparation. Phosphorous content in the root was measured by spectrophometer, and salicylic acid was measured by High Performance Liquid Chromatography (HPLC) according to Chowdappa *et al.* (2013). Roots infected by AM fungi were counted under light microscope after stained with lacto phenol cotton blue.

Field trial

A field test was carried out at the Sukamulya Research Station (S6°56'34.83°; E106°46'21.37"T), Sukabumi, West Java. The field was previously used for conservation of black pepper mother tree. The black pepper vines were prepared with the same ways with the one of green house experiment. The treatments tested were vines inoculated with: (A) *P. fluorescens* (Pf), (B) *Trichoderma* (Tr), (C) *P. fluorescens + Trichoderma* (Pf+Tr), (D) *P. fluorescens+ Trichoderma* + AM fungus (Pf+Tr+AM), and (I) Untreated control. After the treated pepper vines had six to seven nodes, the vines were transplanted on to the field in December 2012, a mid of rainy seasons period of the site.

The pepper vines were planted in five plots for each treatment as repetition, that consist of 16 vines for a single plot. Glyricidia trees were used as living post that planted earlier, and the plant spacing applied was 2.5 m x 2 m, with drainage system (40 cm depth x 60 cm width) among the plots to minimize cross contamination of each treatment. The field observation was ended at December 2014.

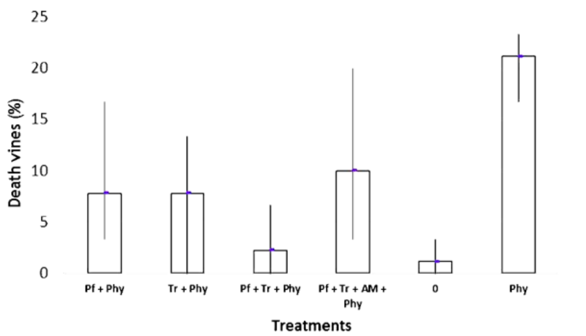
Additional inoculation of *P. fluorescens* and *Trichoderma* were carried out twice a year, at the end and beginning of rainy seasons by drenching the pepper vines with 1 liter *P. fluorescens* suspension (adjusted to 10^8 cfu ml^{-1}) and or 1 liter of *Trichoderma,* adjusted to 10^7 cfu ml^{-1}) or combined with 0.1% molasses of respecting treatment. No additional artificial inoculation for the AM fungus except the one was carried out during seedling preparation. The *Phytophthora* fungus infections were obtained from naturally infection instead of artificial inoculation. All the treated vines were maintained following the standard operational procedure for black pepper, including time and dosages of fertilizer application, limited clean weeding, and pruning the living post (Manohara and Wahyuno, 2013).

The number of dead vines with foot rot symptom were recorded monthly for two years. Regression correlation analysis and Area Under Disease Progress Curve (AUDPC) were performed for describing the dead vines progress and the speed of disease progress (Forbes *et al.,* 2014). The bacterial, fungal and *Trichoderma* population were monitored monthly by diluting the collected soil from each treatment onto the respective medium. The soil samples were collected from the eastern part of the vine, ± 10 cm away from the vines and ± 5 cm depth. Number of spikes were counted at 1.5 m height above the ground, from four vines of different direction branches on each plot of each respective treatment.

RESULTS AND DISCUSSION

Green house test

All tested beneficial microorganisms' application and its combination reduced the foot rot disease incidence after the seedlings were inoculated with *Phytophthora* zoospores suspension artificially. There were no significant differences among the treatments. Numbers of death vines were varied from 3 to 23%, with the highest one was found within the seedlings inoculated with *Phytophthora* individually (Figure 1). The lowest disease incidence was found in the treatments of *Trichoderma* combined with *P. fluorescens* three months after observation (Figure 1).



Note: (Pf) *P. fluorescens,* (Phy) *Phytophthora,* (Tr) *Trichoderma*, (AM) AM fungus, and (0) Untreated/control. Vertical bar represent a value ranged of each treatment.

Keterangan: (Pf) P. fluorescens, (Phy) *Phytophthora*, *(Tr) Trichoderma, (AM) mikoriza arbuskula, (0) Kontrol/tidak diperlakukan. Garis vertikal menunjukkan kisaran nilai dari setiap perlakuan*.

Figure 1. Death vines of pepper of each treatment after artificial inoculation of *Phytophthora,* at seven months old seedlings.

Gambar 1. Tanaman lada yang mati pada setiap perlakuan yang diuji setelah diinokulasi dengan Phytophthora, pada umur tujuh bulan.

Population density of *Trichoderma* in soil media treated with *Trichoderma* individually was relatively lower than others, and the data of control treatment indicated the indigenous *Trichoderma* was exist in soil medium after several weeks, eventhough the soil media was pasteurized prior to use. Total fungal populations density observation revealed no significant differences among the tested treatments, only seedlings inoculated with *Trichoderma* or *Phytophthora* showed high fungal population. For the bacterial population density, only individual inoculation treatment with *P. fluorescens* showed the highest bacterial population (Figure 2). Based on a simple regression analysis, total bacterial and *Trichoderma* population were positively correlated with reducing death vines incidence (data not shown).

The highest phosphorous content on leaves was found on black pepper vines inoculated with *P. fluorescens* individually, but the value was not significantly different as compared to others treatments (Table 1). Salicylic acid contents in the root of each treatment showed that root of pepper vines treated with *Trichoderma* had the highest salicylic acid content. Meanwhile the lowest one was shown by *P. fluorescens* treatment (Table 1). The infected rooting systems by AM fungus was lower than 10% for all treatments after 7^{th} month of cultivation and not significantly different among the treatments (Data not shown).

Inoculation of soil microbial affected to the vegetative growth of black pepper vine up to seven months, however the new sprouting vines percentage was not significantly different among the treatment. The black pepper vines treated with *Trichoderma* produced more nodes than the one treated with *P. fluorescens* individually (Table 1).

The results of the green house experiment indicated the mix application of *P. fluorescens, Trichoderma* and AM fungi were apparently able to compete with artificially inoculated *Phytophthora,* so their populations were steady and improving the plant growth as seen in the increase salicylic acid content (Table 1, Figure 2). Although the applied soil microbes, which give

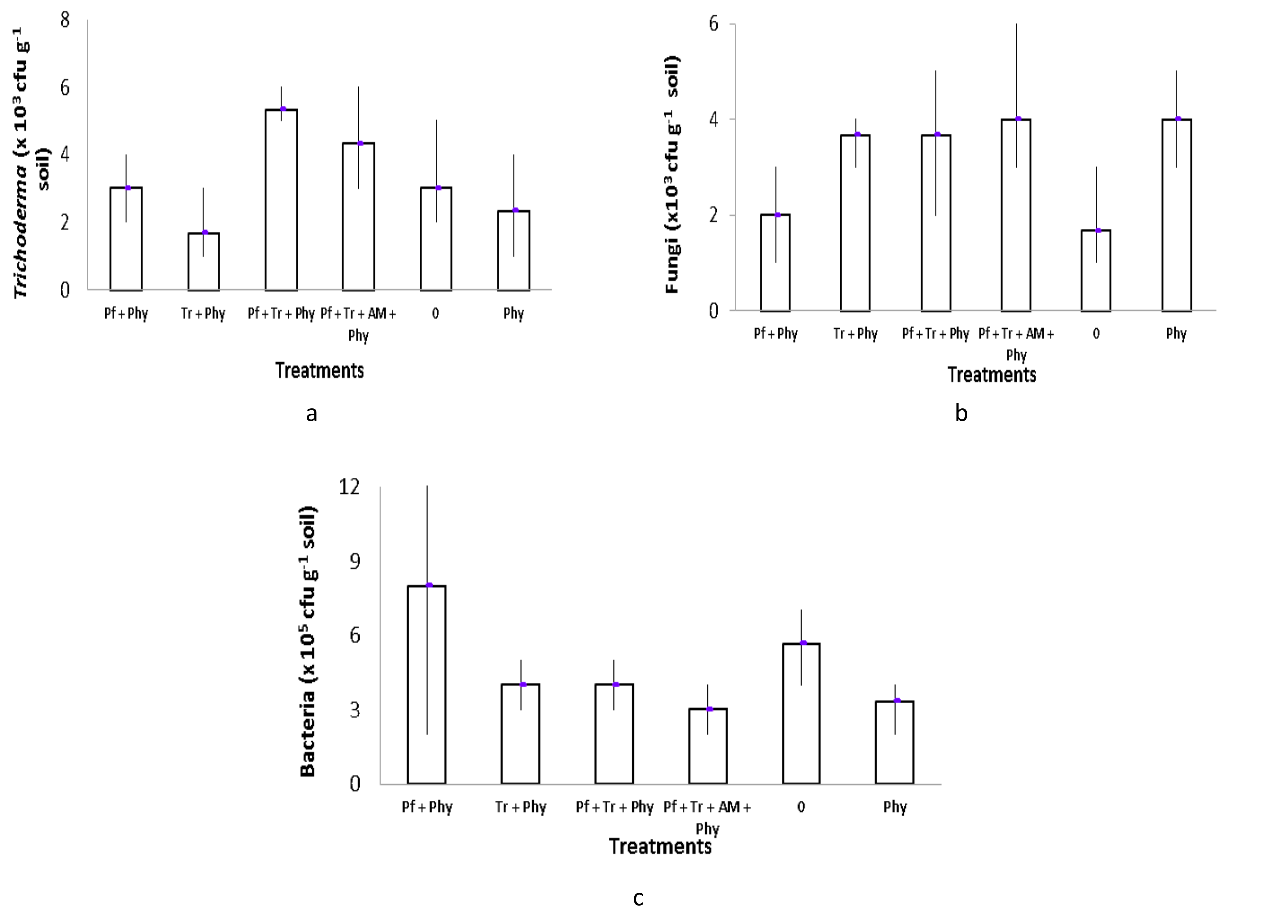


Figure 2. Population density of *Trichoderma,* fungi and bacteria from soil of each treatment at seventh month: (a), *Trichoderma*, (b) Fungi, and (c) Bacteria. Note: (Pf) *P. fluorescens,* (Phy) *Phytophthora,* (Tr) *Trichoderma*, (AM) AM fungus, and (0) Untreated control. Vertical bar represent a value ranged of each treatment.

Gambar 2. Kepadatan populasi *Trichoderma,* total cendawan, dan bakteri pada tiap perlakuan pada bulan ketujuh, setelah perlakuan. Keterangan: (Pf) *P. fluorescens,* (Phy) *Phytophthora*, (Tr) *Trichoderma,* (AM) mikoriza arbuskula, (0) Kontrol tidak diperlakukan, Garis vertikal menunjukkan kisaran nilai dari tiap perlakuan.

Table 1. Phosphorous content in leaf tissue, salisilic acid content in root tissue, sprouting occurrence, node numbers, and root weight of each treatment at 7th month.

Tabel 1. Kandungan fosfor di dalam jaringan daun, asam salisilat pada akar, bibit bertunas, jumlah buku dan berat akar, 7 bulan setelah perlakuan.

Treatments	**Phosphorous in leaf (%)**	**Salicylic acid (%)**	**Sprouting (%)**	**Number of vine nodes**	**Root weight (g)**
Pf + Phy	0,055	0,34	0,91	3,90 a	4,65
Tr + Phy	0,045	0,49	0,84	4,40 ab	3,85
Pf + Tr + Phy	0,050	0,35	0,68	4,20 a	3,66
Pf + Tr + AM + Phy	0,050	0,43	0,69	4,90 bc	3,36
0	0,050	0,35	0,80	6,20 d	3,35
Phy	0,045	0,35	0,81	4,90 c	4,09

Note: (Pf) *P. fluorescens,* (Phy) *Phytophthora,* (Tr) *Trichoderma*, (AM) AM fungus, and (0) Untreated/control.

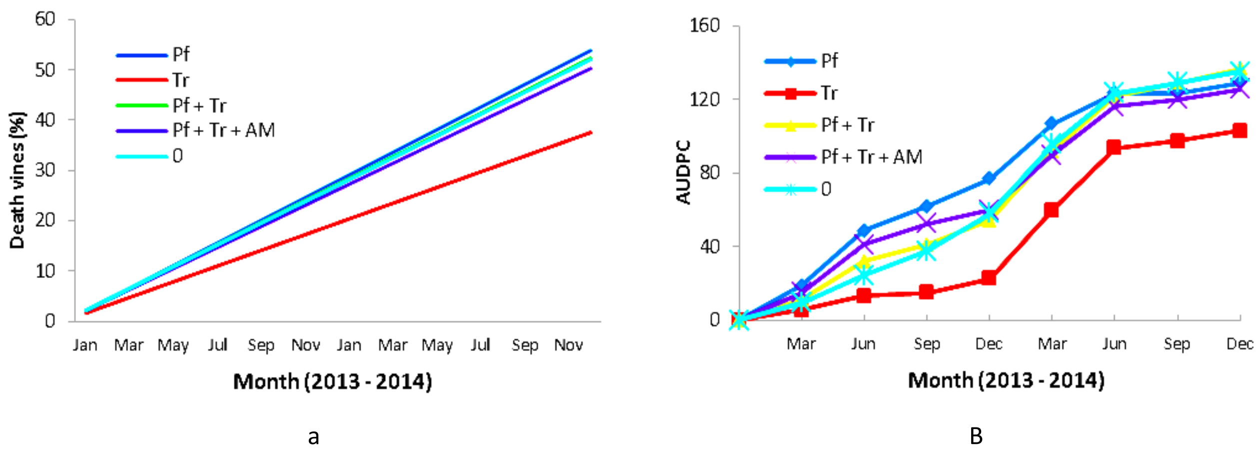
Keterangan: (Pf) P. fluorescens, (Phy) Phytophthora, (Tr) Trichoderma, (AM) mikoriza arbuskula, (E) Kontrol tidak diperlakukan.

significant benefit to pepper vine growth were still unclear. *Trichoderma* spp. and *P. fluorescens* have been reported as inducer for the plant to be resistant against soil borne pathogens, but the compatibility between the host plant and the isolates, and its adaptability to the environment influencing its biocontrol activity effectiveness. Non pathogenic microbes and also biocontrol agents could stimulate the plant resistance against pathogens (Pal and Gardener, 2006). Systemic acquired resistance (SAR) mostly mediated by salicylic acid or jasmine acid, which were produced after pathogens infection, for inducing resistance (Pal and Gardener, 2006). Metabolite, namely 2,4-diacetylphloroglucinol, released by *P. fluorescens* has an important role in stimulating disease resistance of wheat against take all disease (Weller *et al.,* 2002).

Field test

Death black pepper vines were found at the first two months after transplanting, for all tested treatments. The foot rot disease incidence of individual plots was lower for *Trichoderma* application as compared to others. These patterns had been shown for two years until the observation was terminated on December 2014. There was no significant different among the tested treatments statistically, but all treatments showed linear growing patterns-fitness which were typical to soil borne disease's progress (Figure 3).

AUDPC analysis also showed that the foot rot disease progress was lower for individually *Trichoderma* treatment. The AUDPC values of all tested treatments were higher during the dry seasons and the AUDPC curve tended to increase as the time observation continued monthly, and the*Trichoderma* treated plot was the lowest one (Figure 3). Final accumulation of AUDPC value of each treatment after observation for two years were *P. fluorescens* (690.0), *Trichoderma* (410.6), *P. fluorescens* and *Trichoderma* (618.8), *P. fluorescens, Trichoderma* and AM fungus (620.6), and control (613.1).



Note: (Pf) *P. fluorescens*, (Tr) *Trichoderma,* (AM) AM fungus, and (0) Untreated/control.

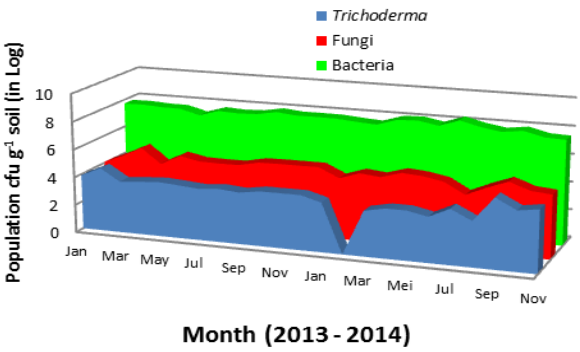
Keterangan: (Pf) P. fluorescens, (Tr) Trichoderma, (AM) mikoriza arbuskula, dan (0) Kontrol/tidak diperlakukan.

Figure 3. The death vines curve of black pepper within two years in the treated plots. (a) in linear format, and (b) in AUDPC format.

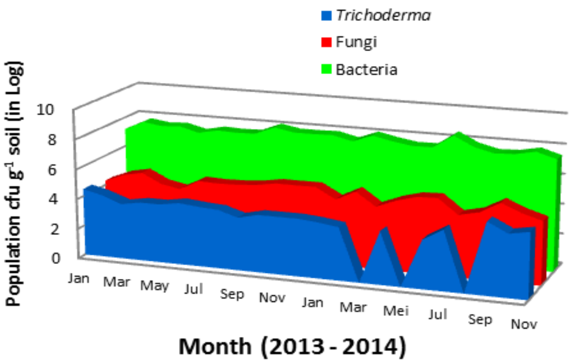
Gambar 3. Perkembangan jumlah tanaman lada yang mati pada setiap perlakuan selama dua tahun pengamatan. (a) Bentuk persamaan linear, dan (b) Bentuk Area Under Progress Curve.

Population of *Trichoderma,* total fungi and total bacteria in the soil of each treatment indicate that all treatments showed the same patterns, to which those two groups of microbes were exist throughout the observation. There was no sharply fluctuation for population density of both total fungi and bacteria. However, *Trichoderma* population density was varied among the treatments. *Trichoderma* populations were not detected during the dry season, of March, May, June and August, 2014 (Figure 4).

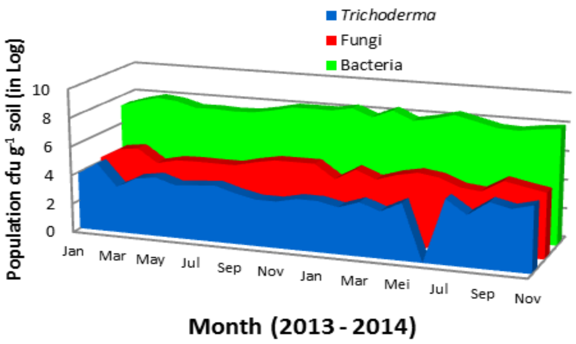
At the end of observation, the spikes started to form, and at individual *Trichoderma* treatment plots, the spikes were formed more



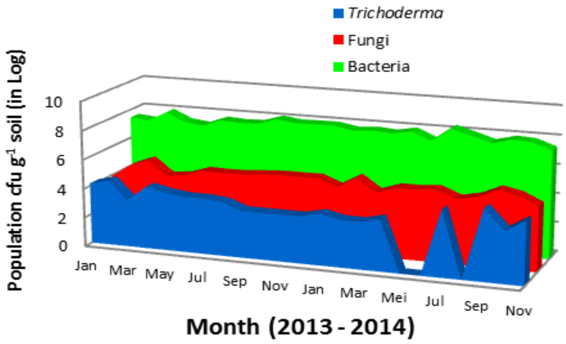
Pf



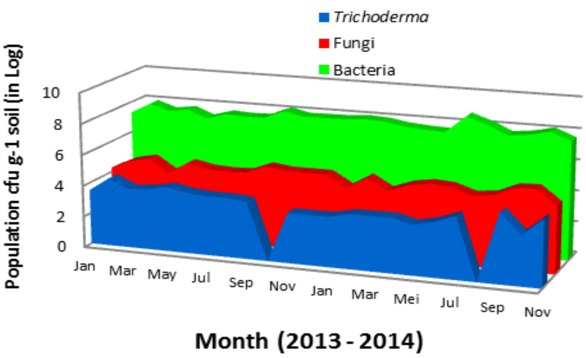
Pf + Tr + AM



Tr



0

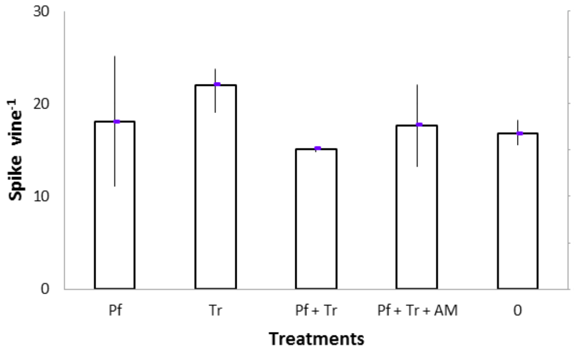


Pf + Tr

Note: (Pf) *P. fluorescens*, (Tr) *Trichoderma,* (AM) AM fungus, and (0) Untreated/control.
Keterangan: (Pf) P. fluorescens, (Tr) Trichoderma, (AM) mikoriza arbuskula, dan (0) Kontrol/tidak diperlakukan.

Figure 4. Population density of *Trichoderma,* fungi and bacteria in soil of each tested treatment in two years.
Gambar 4. Kepadatan populasi Trichoderma, total cendawan, dan bakteri pada tiap perlakuan selama dua tahun.

figures than others (Figure 5). The time when the experiment was terminated, the pepper vines just started flowering for the first time.



Note: (Pf) *P. fluorescens*, (Tr) *Trichoderma,* (AM) AM fungus, and (0) Untreated control. Vertical bar represent the ranged value of each treatment.

Keterangan: (Pf) P. fluorescens, (Tr) Trichoderma, (AM) mikoriza arbuskula, dan (0) Kontrol tidak diperlakukan. Garis vertikal menunjukkan kisaran nilai dari tiap perlakuan.

Figure 5. Number of spikes of each tested treatment at 1.5 m height.

Gambar 5. Jumlah malai yang terbentuk pada tiap perlakuan pada ketinggian 1,5 m dari permukaan tanah.

In the field test, only individual application of *Trichoderma* be able to reduce the foot rot disease incidence. Whereas other treatments showed similar results with control, up to two years' observation (Figure 3). The result was differed with the green house experiment. Complex interaction among the applied beneficial soil microbes with the existing soil microbes in the natural soil might influence the results of the field test. *Phytophthora* is an opportunistic plant pathogenic fungus with a low competitiveness ability to survive as saprophytic microbes. However, the fungus is able to parasite and devastate the pepper vines quickly under conducive condition. The zoospore of the fungus is an important agent for disease dispersal and infection (Manohara *et al.,* 2004). Individual application of *P. fluorescens* on pepper seedling effectively reduced foot rot disease incidence up to 50% at 60 days after planting in India (Anith *et al.,* 2002). Strains of *P. fluorescens* produced β-1, 3 and β-1, 4 glucanase, and lipases be able to lytic mycelia *P. capsici* of black pepper when they were cultured together (Diby *et al.,* 2005), and also mycelia of *Rhizoctonia* and *P. capsici* of tomato (Saad, 2006). Chowdappa *et al.,* (2013) reported that *Trichoderma* was able to control and promote induce systemic resistance in tomato against *Phytophthora* and *Alternaria.* Combination of *Trichoderma* and *Pseudomonas* induced systemic resistance significantly against *Fusarium* in *Arabidopsis* (Alizadeh *et al.,* 2013). Therefore, improving beneficial soil microbial community population in soil prior to infection of the plant pathogenic fungi is a crucial period in biological control of soil borne diseases.

In the present study, black pepper vein inoculated with a single microbe, *Trichoderma* can be survived and adapted to current environment which were shown positive results in reducing foot rot disease incidence. This experiment indicated that application of beneficial microbial, which were able to compete and adapt to the natural environment was important in reducing disease occurrence. A competion among beneficial microorganisms in mixing application may also happened. The metabolites substances produced by biocontrol bacteria or fungi that contribute to their biocontrol activity against pathogenic fungus have also influenced to other beneficial microorganisms. The competition among a mixture of different promising isolates of *Trichoderma* might happened and caused nullified their individual effect in reducing foot rot disease incidence of black pepper (Rajan *et al.,* 2002). Duffy *et al.* (1996) suggested to apply the beneficial microorganisms in separation to have the effectiveness of its compatibility in suppressing disease incidence. Mostly diseases suppression of soil borne diseases in agricultural field was caused by the existing biological activity process (Weller *et al.,* 2002). Competition in nutrient, antagonism, amensalism, parasitism and systemic acquired resistance were mechanism might involved in disease suppression (Gerbeva *et al.,* 2004). Competition between pathogenic and non-pathogenic microbes for soil nutrient consumption was an important aspect in limiting disease incidence and severity occurrence (Pal and Gardener, 2006). Cultural practices such as application of organic matters increase population and activity of soil microbes, which leads the disease suppression (Weller *et al.,* 2002). Crop rotation was needed to promote the biocontrol activity of the applied beneficial microbes by changing the interaction and competition in the soil environment, sometime (Larkin, 2008); organic substrates with sufficient amount is important in sustaining and succeed in the biocontrol activity process against soil borne pathogenic fungi (Hoitink and Boehm, 1999).

The applied AM fungus seems not significant in reducing disease incidence in the present study, probably due to the unsuitability of planting medium for AM to perform well. Thus, the effect of AM fungus on plant vigor was not obviously clear either in the greenhouse or the field trial. Though the fungus were collected from black pepper rhizosphere (Trisilawati and Rochmat, 2005).

The present study indicated that in natural condition, artificial reinoculation of beneficial microorganisms is regularly needed to maintain the applied beneficial microbe population steady, and performing its potential biological activity to run optimally. The application of appropriate *Trichoderma* strains is an important factor for reducing foot rot disease incidence of black pepper, since the fungus relatively well adapted in the wide agro ecological zone. Screening indi-genous *Trichoderma* from each respecting black pepper developing areas may need to be considered by the existing local field station in the future.

CONCLUSION

The application of *Trichoderma, P. fluorescens* and their combination reduced foot rot disease incidence of black pepper in the green house experiment, but in the natural condition only the application of *Trichoderma* individually reduced the disease incidence.

Application of *Trichoderma* promoted salicyclic acid formation in the root. Regular application of *Trichoderma* is important in maintaining the population steady, and reducing foot rot disease incidence in black pepper.

ACKNOLEDGEMENT

Acknoledgement addressed to Dr. Sarma YR. FAO consultant for invaluable suggestion. Gratitudes also addressed to Sutrasman, Zulhisnain, U Mansyur, Y Hidayat and N Karyani for wondeful technical assistant during the study. This study was funded by Indonesian Government through ISMCRI.

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