Effect of *Trichoderma* spp. and potassium fertilizer on *Phytophthora palmivora* infection in cacao seedlings

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**Abstract.** *Phytophthora palmivora* is a pathogen that causes black pod, leaf blight, and stem cancer on cacao, that cause yield loss up to 40%. Control of *P. palmivora* is intended to make plants healthy, by using *Trichoderma* spp. and potassium fertilizer. The study was aimed to determine the effect of *Trichoderma* spp. and potassium fertilizer application against *P. palmivora* infection in cacao seedlings. The study used a randomized block design consisting of 13 treatments with 5 replications. The treatments were *Trichoderma* spp. (*T. atroviride* JB2, *T. amazonicum* LP3, *T. hamatum* LP2), and potassium fertilizers dosages (1, 2 and 3 g), chemical fungicides (mancozeb) for comparison, and control. Cacao seedlings were treated with *Trichoderma* spp. and potassium fertilizer, then the plants were inoculated with *P. palmivora*. Observations were on attack symptoms, incubation period, disease severity, growth of cacao seedlings, and lignin content. The results showed that the application of *Trichoderma* spp. and potassium fertilizer prolonged the incubation period and reduced the number of leaves spots (48.95–75.37%). *T. amazonicum* + 3 g of potassium fertilizer and *T. hamatum* + 2 g of potassium fertilizer suppressed the number of spots up to 75.37% and 71.36% respectively, higher than chemical fungicides that were 70.86%.

Keywords: cacao, *P. palmivora* infection, *Trichoderma* spp., potassium fertilizer, suppression.

**Introduction**

Cacao (*Theobroma cacao* L.) is a plantation commodity that has an important role in the Indonesian economy, both as a source of farmers’ income and as an export commodity. Indonesia is one of the largest cacao producing countries, with an area of 1,774,500 ha and the production of 777,500 tons of dry beans, after Ivory Coast and Ghana [1]. Cacao productivity in Indonesia in 2014 reached 817 kg/ha [2], but these results have not been able to meet the needs of domestic industries, so Indonesia also became an importer of cocoa beans at 46.075 tons/year.

The problem faced by national cacao is low productivity and quality. The low productivity and quality of cacao farmers are due to major diseases attack among which black pod caused by *Phytophthora palmivora* [3,4]. As a result, pod disease may cause an average of 20–40% yield loss, and may be worse in areas with high rainfall and humidity [5,8]. In Indonesia, the yield loss may be as high as 100%, especially during the rainy season [9]. *P. palmivora* is difficult to control because the fungus survives in the form of mycelium and chlamydospora on infected plant parts or in the soil. Pruning and...
burying the infected pod into the soil is recommended for minimizing source of inoculum [7], but the results have not been satisfactory.

Current control is directed at how to make plants healthy, especially by using biological control agents (BCAs) and fertilization. Among BCAs that have the potential to be developed are *Trichoderma* spp. Some *Trichoderma* species, namely *T. viride*, *T. harzianum*, *T. virens*, *T. hamatum*, *T. koningii*, and *T. polysporum* have been widely used to control various plant diseases [10-12].

*T. martiale* has been reported potential to control black pod caused by *P. palmivora* [13]. *T. asperellum* also has been reported reduce black pod up to 50% [14]. *T. virens* to control black pod in cacao, the results reported that BCAs can suppress disease intensity up to 71%, and *T. virens* formulation with the addition of corn oil, was better at suppressing black pod compared to other organic oils [11, 15].

In addition to the application of BCAs (*Trichoderma* spp.), applying fertilizers, especially potassium fertilizer will also improve plant health. Potassium fertilizer helps the development of roots, the formation of proteins, carbohydrates, and increases plant resistance to disease. Generally, plants lacking in potassium elements will disrupt the resistance component so that it will facilitate pathogens for penetration [16], whereas potassium fertilizers reduce disease attacks, which are related to pathogen penetration [17]. The application of potassium fertilizer 3 g increases rubber plant resistance to infectious *Corynespora* leaf fall [18]. Meanwhile, other reported that potassium fertilizer 3 g/plant reduces the intensity of downy mildew *Peronosclerospora maydis* [16].

The study aimed to determine the effect of *Trichoderma* spp. and potassium fertilizer on *P. palmivora* infection in cacao.

**Materials and methods**

The research was conducted at the Plant Protecion Laboratory and Greenhouses of the Indonesian Industrial and Beverage Crops Research Institute (IIBCRI), Sukabumi, from April to November 2017.

1.1. Isolation and multiplication of pathogens

*P. palmivora* was isolated from the infected pod. The surface of the pod was sterilized with moistened tissue paper with 70% alcohol. A piece (± 0.5 cm²) of pod flesh was taken from the infected tissue, then planted into 2% water agar (WA) medium and incubated for 4 days at room temperature. After 4 days, the growing fungal colonies were subculutred and purified on agar V8 media (200 ml of V8 juice, 800 ml of aquades, 1 g of CaCO₃, and 20 g of bacto agar) and identified using the standard protocol for identification of *P. palmivora* [19]. The obtained *P. palmivora* PL isolate [20] was prepared for the inoculum by growing the isolate on the V8 agar medium for mass multiplication and incubated at room temperature for 6 days.

1.2. Regeneration of *Trichoderma* spp.

*Trichoderma* spp. isolates in this study is a culture collection from the IIBCRI which have been tested and potential to suppress plant pathogenic fungi [21–23]. The three isolates were *T. hamatum* LP2, *T. atroviride* JB2, *T. amazonicum* LP3 with optimum conidial density of 10⁸ spores/ml were used for the current study [21–23]. *Trichoderma* spp. isolates were regenerated on selective a medium (3 g glucose, 1 g NH₄NO₃, 0.9 g KH₂PO₄, 0.2 g MgSO₄, 0.15 g KCl, 0.02 g ZnSO₄, 0.02 g MnSO₄, 0.15 g Rose Bengal, 0.25 g of chloramphenicol, 0.05 g of streptomycin sulfate, and 20 g of bacto agar). Then, they were multiplicated on a PDA medium.

1.3. Mass production of *Trichoderma* spp.

Mass production of *Trichoderma* spp. carried out using a simple fermentor series, consisting of gallons for fermentation medium, aerators for air supply and 0.2 μm filters to filter air. The fermentor and medium were sterilized in an autoclave at 121 °C for 15 minutes. The composition of medium are glucose 20 g; potato 100 g; Na₂HPO₄ 3.4 g; NaH₂PO₄ 1.99 g; urea 1 g; KCl 0.2 g; MgSO₄ 0.2 g; Thiamin
HCl 1 mg; MnSO₄ 0.002 g; ZnSO₄ 0.002 g; FeSO₄ 0.002 g; 1,000 ml RO water. Furthermore, in the fermentative media, vegetative inoculums were added as much as 2% [v/v], then incubated at a temperature between 25 and 28 °C for 4–5 days. One ml of suspension [1% (v/v)] was pipeted into a 500 ml Erlenmeyer which had been filled with 100 ml vegetative media and was incubated at 25–28 °C for 3 days on an orbital shaker with 150 rpm speed.

1.4. Testing of Trichoderma spp. and potassium fertilizer against P. palmivora infection in cacao seedlings

*P. palmivora* is not only infecting cacao pod but also the seeds and stems. Testing of *Trichoderma* spp. and potassium fertilizers carried out in greenhouses of IIBCRI with local cacao seedlings that are susceptible to *P. palmivora* as testing plant. *Trichoderma* spp. isolates used were the three best isolates from based on in vitro observation, while the potassium fertilizer used at different doses. The experiment was arranged in a randomized block design. The treatments were species of *Trichoderma* spp. (T. hamatum LP2, T. atroviride JB2, and T. amazonicum LP3), and potassium fertilizer dosage (1, 2, and 3 g), with 5 replications for each treatment (Table 1). The used cacao seedlings were 4 months old hybrid seeds, planted in polybags containing soil : manure (2:1).

Cacao seedlings were treated with *Trichoderma* spp. by spraying conidial suspension (10⁸ conidia/ml) throughout the leaf surface. The potassium fertilizer was applied around the roots, one month before application of *Trichoderma* spp. Then the seeds were covered with a plastic bag to keep moisture. One week after *Trichoderma* spp. application, cacao seedlings were inoculated with *P. palmivora* by spraying the zoospore suspension (10⁶ spores/ml) onto all the leaves [11], then covered again with the plastic bag. As a control treatment, cacao seedlings were inoculated by *P. palmivora* without *Trichoderma* spp. As a comparison using a chemical fungicide (mancozeb), with recommendation dosage.

Observations were carried out on the development of disease symptoms and the number of spots on the cacao seedlings that were conducted every day to 3 months. Observations were also on lignin levels content in leaf tissue. Analysis of lignin levels was carried out at the Biotechnology Laboratory, the Agency for the Assessment and Application of Technology, Serpong, using Klason method [24].

| No. | Treatments                      | dosage 1 g/plant | dosage 2 g/plant | dosage 3 g/plant |
|-----|---------------------------------|------------------|------------------|------------------|
| 1.  | *T. hamatum* LP2                | T1D0             | T1D1             | T1D2             |
| 2.  | *T. atroviride* JB2             | T2D0             | T2D1             | T2D2             |
| 3.  | *T. amazonicum* LP3             | T3D0             | T3D1             | T3D2             |
| 4.  | Without *Trichoderma* spp.     | D0               | D1               | D2               |
| 5.  | Chemical fungicide              | F                | -                | -                |

1.5. Analysis of data

The obtained data were analyzed for a variance and had differences occurred then followed by DMRT test at the level of 5%.

Results and discussion

1.6. Incubation period and disease severity

Attack symptoms on the leaves showed yellow spots, then the spots enlarge and coalesce with neighboring spot to form wide necrotic lesions on the infected leaf. The infected plants will wither when the pathogens attack the shoots (Figure 1).
Figure 1. Symptoms of *P. palmivora* attack on cacao leaves: early symptom (a) and development symptom (b)

Table 2. Effect of *Trichoderma* spp. and potassium fertilizer on the incubation period, number of spots, and suppression of the number of *P. palmivora* spots on cacao leaves

| Treatments | Incubation period (days) | Number of spots | Suppression of the number of spots (%) |
|------------|--------------------------|-----------------|---------------------------------------|
| T1D0 (*T. atroviride* + 1 g potassium fertilizer) | 5.75<sup>ab</sup> | 14.55<sup>b</sup> | 51.04 |
| T1D1 (*T. atroviride* + 2 g potassium fertilizer) | 6.12<sup>a</sup> | 9.00<sup>cd</sup> | 69.71 |
| T1D2 (*T. atroviride* + 3 g potassium fertilizer) | 5.07<sup>ab</sup> | 11.50<sup>c</sup> | 61.30 |
| T2D0 (*T. hamatum* + 1 g potassium fertilizer) | 5.67<sup>ab</sup> | 14.47<sup>b</sup> | 51.31 |
| T2D1 (*T. hamatum* + 2 g potassium fertilizer) | 6.14<sup>a</sup> | 8.50<sup>d</sup> | 71.36 |
| T2D2 (*T. hamatum* + 3 g potassium fertilizer) | 5.63<sup>ab</sup> | 12.66<sup>bc</sup> | 57.40 |
| T3D0 (*T. amazonicum* + 1 g potassium fertilizer) | 5.22<sup>ab</sup> | 14.35<sup>b</sup> | 51.71 |
| T3D1 (*T. amazonicum* + 2 g potassium fertilizer) | 5.20<sup>ab</sup> | 13.32<sup>bc</sup> | 55.18 |
| T3D2 (*T. amazonicum* + 3 g potassium fertilizer) | 6.10<sup>a</sup> | 7.32<sup>d</sup> | 75.37 |
| FD0 (mancozeb + 1 g potassium fertilizer) | 4.90<sup>b</sup> | 13.50<sup>bc</sup> | 54.57 |
| FD1 (mancozeb + 2 g potassium fertilizer) | 6.40<sup>a</sup> | 14.42<sup>b</sup> | 51.48 |
| FD2 (mancozeb + 3 g potassium fertilizer) | 5.50<sup>ab</sup> | 8.66<sup>d</sup> | 70.86 |
| K (control) | 4.20<sup>b</sup> | 29.72<sup>a</sup> | |

Notes: Numbers followed by the same letters in each column are not significantly different according to DMRT test at 5% levels

Results showed that *Trichoderma* spp. and potassium fertilizer prolonged the incubation period of *P. palmivora* infection in cacao leaves compared to control. The fastest incubation period in the control treatment was 4.2 days and the longest in FD1 (mancozeb + 2 g potassium fertilizer), which was 6.40 days. No different with T2D1 (*T. hamatum* + 2 g potassium fertilizer) was 6.14 days, followed by T1D1 (*T. atroviride* + 2 g potassium fertilizer) and T3D2 (*T. amazonicum* + 3 g potassium fertilizer), 6.12
days and 6.10 days, respectively. Meanwhile, the incubation period of \textit{P. palmivora} infection in other treatments ranged from 4.64 to 5.75 days (Table 2).

Long incubation period at T2D1 treatment (\textit{T. hamatum} + 2 g potassium fertilizer); T1D1 (\textit{T. atroviride} + 2 g potassium fertilizer); T3D2 (\textit{T. amazonicum} + 3 g potassium fertilizer) caused by \textit{Trichoderma} spp were able to inhibit the germination of zoospores from \textit{P. palmivora}. Furthermore, potassium fertilizers increase plants resistance response so that the penetration of \textit{P. palmivora} can be blocked. Potassium nutrient influences the mechanism of stomata opening and closing [25]. Therefore, potassium in sufficient quantities reduces the penetration of disease through stomata and increases leaf area so that photosynthetic capacity is better in leaves with a larger area compared to deficient plants. The potassium element also increases epidermal thickness so that it inhibits pathogen penetration.

Results of the number of \textit{P. palmivora} spots revealed that potassium fertilizer and \textit{Trichoderma} spp reduced the number of spots compared to control (Table 2). The lowest spots number was observed in T3D2 (\textit{T. amazonicum} + 3 g potassium fertilizer) i.e 7.32, not different from FD2 (mancozeb + 3 g potassium fertilizer) i.e 8.66, followed by T2D1 (\textit{T. hamatum} + 2 g potassium fertilizer) 8.50 and T1D1 (\textit{T. atroviride} + 2 g potassium fertilizer) 9.00 with suppression on the spots of 75.37%, 71.36%, 70.86% and 69.71%, respectively. In other treatments, the number of spots ranged from 11.21 to 14.87 with suppression on the number of spots was 49.96–62.68%. The high suppression of \textit{P. palmivora} spot numbers was observed in T3D2 (\textit{T. amazonicum} + 3 g potassium fertilizer). This may caused by ability of \textit{T. amazonicum} producing secondary metabolite compounds that induce plant resistance by increasing salicylic acid [26]. \textit{T. hamatum} suppressed \textit{P. palmivora} infection because the fungus induced plant resistance by increasing phenol compounds content [20].

\textbf{1.7. Growth of cacao seedlings}

Effect of \textit{Trichoderma} spp. (\textit{T. atroviride} JB2, \textit{T. hamatum} LP2, and \textit{T. amazonicum} LP3) and potassium fertilizer dosages (1, 2, and 3 g) on cacao seedlings growth in greenhouses, showed that potassium fertilizer with all dosages increase plant growth (plant height, number of leaves, and stem diameter). The increase in plant height ranged from 7.99 to 20.88 cm, the largest increase was observed in T1D1 treatment (\textit{T. atroviride} + 2 g potassium fertilizer) 20.83 cm, followed by T2D1 (\textit{T. hamatum} + 2 g potassium fertilizer), T1 (\textit{T. atroviride}), 20.50 cm and 20.44 cm, respectively, while the increase in other plant height ranged from 10.05 to 18.89 cm and the smallest was observed in T3D0 (\textit{T. amazonicum} + 1 g potassium fertilizer) 7.99 cm (Figure 2).
Figure 2. Effect of *Trichoderma* spp. and potassium fertilizer to increase plant height, number of leaves, and stem diameter.

T1D0 (*T. atroviride* + 1 g potassium fertilizer), T1D1 (*T. atroviride* + 2 g potassium fertilizer), T1D2 (*T. atroviride* + 3 g potassium fertilizer), T2D0 (*T. hamatum* + 1 g potassium fertilizer), T2D1 (*T. hamatum* + 2 g potassium fertilizer), T2D2 (*T. hamatum* + 3 g potassium fertilizer), T3D0 (*T. amazonicum* + 1 g potassium fertilizer), T3D1 (*T. amazonicum* + 2 g potassium fertilizer), T3D2 (*T. amazonicum* + 3 g potassium fertilizer), FD0 (*mancozeb* + 1 g potassium fertilizer), FD1 (*mancozeb* + 2 g potassium fertilizer), FD2 (*mancozeb* + 3 g potassium fertilizer), K (control)

Results indicated that effect of *Trichoderma* spp. and potassium fertilizer on the number of leaves in cacao seedlings varied. The largest number of leaf growth was treated by FD2 (*mancozeb* + 3 g potassium fertilizer), i.e. 12.22 leaves, followed by T3D0 (*T. amazonicum* + 1 g potassium fertilizer), FD0 (*mancozeb* + 1 g potassium fertilizer), T1 (*T. atroviride*), T0D1 (2 g potassium fertilizer), FD1 (*mancozeb* + 2 g potassium fertilizer), T3D2 (*T. amazonicum* + 3 g potassium fertilizer) ie 10.88; 1077; 10.25; 10.11 leaves, respectively. *Trichoderma* spp. and potassium dosage application had not a significant effect in increasing stem diameter growth. Increased stem diameter among treatments were ranges from 0.03–0.17 cm (Figure 2). Potassium fertilizer can help the development of roots, formation of proteins and carbohydrates, increase plant resistance to disease and drought [27].

1.8. Lignin content

Application of *Trichoderma* spp. combined with potassium fertilizer increased the lignin content in the leaves. T1D2 (*T. atroviride* + 3 g potassium fertilizer) and T3D1 (*T. amazonicum* + 2 g potassium fertilizer) treatment has a higher lignin content compared to control (6.88% and 5.72%, respectively), whereas T2D2 was similar with the one of control. FD1 (*mancozeb* + 2 g potassium fertilizer) and FD2 (*mancozeb* + 3 g potassium fertilizer) can also increased lignin content higher than control.
Figure 3. Effect of *Trichoderma* spp. and potassium fertilizer on lignin content in cacao leaves.

T1D0 (*T. atroviride* + 1 g potassium fertilizer), T1D1 (*T. atroviride* + 2 g potassium fertilizer), T1D2 (*T. atroviride* + 3 g potassium fertilizer), T2D0 (*T. hamatum* + 1 g potassium fertilizer), T2D1 (*T. hamatum* + 2 g potassium fertilizer), T2D2 (*T. hamatum* + 3 g potassium fertilizer), T3D0 (*T. amazonicum* + 1 g potassium fertilizer), T3D1 (*T. amazonicum* + 2 g potassium fertilizer), T3D2 (*T. amazonicum* + 3 g potassium fertilizer), FD0 (mancozeb + 1 g potassium fertilizer), FD1 (mancozeb + 2 g potassium fertilizer), FD2 (mancozeb + 3 g potassium fertilizer), K (control)

Conclusion

Application of *Trichoderma* spp. and potassium fertilizer prolonged the incubation period and reduced the number of *P. palmivora* spots on cacao leaves up to 48.95–75.37%. The highest suppression of spots was *T. amazonicum* with 3 g potassium fertilizer and *T. hamatum* with 2 g potassium fertilizer (75.37% and 71.36%, respectively) higher than mancozeb, which was 70.86%. *Trichoderma* spp. and potassium fertilizer application also increased the growth of cacao seedlings.

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