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Development of *Pratylenchus coffeae* in Biochar Applied Soil, Coffee Roots and Its Effect on Plant Growth

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Abstract

Biochar improve physical, chemical and biological properties of soil. It may also be used as botanical pesticides. The experiment was aimed to determine the effect of biochar on population development of parasitic nematode *Pratylenchus coffeae*. The experiment was carried out in Nematology Laboratory and in a greenhouse of Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, Indonesia. The experiment was arranged according to completely randomized design with six treatments of biochar concentrations, i.e. 4%; 3%; 2%; 1%; 0.5% and 0% dry weight of soil. Research results revealed that biochar application of 4.0% was effectively killed 74.5% of *P. coffeae*, while the lowest mortality level of 37.5% at biochar 0.5% treatment. In the greenhouse trial, application of biochar 4.0% was the most effective in suppressing the population of parasitic nematode in coffee seedlings. Application of biochar was also able to increase the growth and biomass of coffee seedlings.

Keywords: organic matter, biochar, coffee, parasitic nematode, *Pratylenchus coffeae*

INTRODUCTION

Parasitic nematodes have become serious issues for coffee plantation. One important parasitic nematodes infecting coffee plants, particularly Arabica coffee, is *Pratylenchus coffeae*. Until recently, some methods have been implemented to control the parasitic nematode and one of them is nematicide. Beside expensive, nematicides also may damage the environment and other organisms. Therefore, it is important to develop an environmentally and friendly alternative to control the nematode, such as by using organic substances. Improving soil organic matter content has been known to reduce the parasitic nematodes. Wiryadiputra (1995) had demonstrated that organic matters (e.g. manure, coffee husks, and compost) reduced approximately 70% of the parasitic nematode population. However, applied soil organic matter decompose quickly, eventually soil organic matter in soil decrease. Biochar has an ability to increase organic matter content in soil for a long time. According Qoyyum *cit*. Pujiyanto (2013), applied biochar may stabilize soil organic matter content in long term.

Biochar is one of soil revitalization material made from various types and forms of biomass including agricultural, forestry and plantation wastes such as pieces of wood, coconut shells, corn cobs, rice/bean husks, timber waste, sugarcane industrial wastes and other recyclable organic materials. Generally, the organic materials are burned incompletely or without oxygen, and most frequently using external heat source (pyrolysis).

Biological charcoal formed from the heating process will produce activated carbon which contains calcium, magnesium and inorganic carbon (Lehmann & Rondon, 2006).

Crop residues derived from agricultural, forestry and plantation wastes are relatively cheap potential sources of biomass energy. Most of these materials are no longer used and therefore, more affordable compared to other materials that were deliberately processed as materials for biomass (Horgan *cit*. Gani, 2009). Biochar can improve physical, chemical and biological characteristics of soil, including improving capacity of the soil to hold more water in order to reduce run-off and leaching of nutrients, increasing soil pH, cation exchange capacity (CEC), organic C, total N and reducing activity of Fe and Al ions resulting in increasing P content, increasing bacterial population, and composition, also activity of enzymes around plant roots (Rondon & Lehmann, 2007; Wang *et al*., 2012).

Besides improving physical, chemical and biological characristics of soil, biochar can also function as a pesticide. The findings of Matondang *et al*. (2014) showed that application of 20 g coconut shell charcoal per plant reduced 32.3% of the disease caused by *Phytophtora nicotianae* in tobacco compared to control. Palm shell charcoal also reduced *Fusarium oxysporum* infection in chilli plants to 9.31%; the percentage was lower than control (15.4%) (Sinaga, 2011). Therefore, it is possible that biochar is able to reduce parasitic nematodes in coffee plantation. The purpose of this study was to investigate the effectiveness of biochar as a pesticide in controlling parasitic nematodes, particularly *P. coffea* both in soil and coffee roots and also on plant growth, and eventually can be used as an environmental-friendly control in coffee plantations.

MATERIALS AND METHODS

Biochar used in this study derived from burned log of woods. The biochar was ground and sieved into powder that could pass through ±5 mm sieve. *Pratylenchus coffeae* nematodes were obtained from extraction of coffee infected by *P. coffeae* from Kaliwining Experimental Station of ICCRI, Jember, Indonesia. Improved Baermann method (Viglierchio & Schmitt, 1983) was applied for the extraction so that the nematodes were still alive and healthy.

In vitro study was conducted in the Nematology Laboratory of ICCRI, Jember. The experiment was arranged based on completely randomized design with six treatments and each treatment was replicated four times. The treatments were 0.5%, 1%, 2%, 3%, and 4% biochar concentration per 10 mL of water as well as 0% (water without biochar as control). The *P. coffeae* nematodes were put into petridishes (diameter 9 cm) containing 10 mL of water. The biochar with different concentration was then added to the petridish. There were 50 nematodes in each petridish (single treatment), regardless male-female ratio of the organisms. Observations were conducted on the *P. coffeae* mortality in 24 hours after the treatment. Level of efficacy was calculated based on the Schneider-Orelli's formula (Ciba-Geigy, 1981), as follows:

$$\% \text{ Efficacy} = \left(\frac{\% \text{ MT} - \% \text{ MC}}{100 - \% \text{ of MC}}\right) \times 100$$

where MT (mortality in treatment) and MC (mortality in control).

In vivo study was conducted to understand the influence of biochar on *P. coffeae* population and on growth of coffee plants and was conducted in a glass house of ICCRI. This study used completely randomized design. The treatments were 0.5%, 1%, 2%, 3%, and 4% biochar concentration based on dried soil as well as 0% (no biochar as control). The treatments were replicated four times. One week after *P. coffeae* nematode inoculation, the biochar, according to each treatment, was poured around the coffee plants and covered ±5 cm below the soil snoface.

Coffee seedlings used were the butterfly stage (two pairs of leaves) of S 795 Arabica coffee variety planted in polybags with planting medium volume of 300 mL. The planting medium was a mixture of fine sand and manure with ratio of 2:1. The planting medium had been sterilized using an autoclave at the pressure of 2 atm at 120^{O}C for four hours. Two weeks after the coffee seedlings was transfered into polybags, the seedlings were inoculated with 50 parasitic nematodes/ polybag and planted in 10 cm depth hole.

The focus of the observation was growth of the coffee seedlings which involved plant height, number of leaves, weight of fresh roots, weight of fresh and dried shoot as well as population of the nematodes at the end of the observation (three months after the application of biochar). Plant height and leaf number were observed for three months. The population of the nematodes was observed at the end of the observation (in soil sample and root tissue). The centrifuse method was conducted for extracting the nematodes. The infection of the nematodes was evaluated based on the percentage of root lesion or root rot. Data was analyzed using Duncan's multiple range test (DMRT) at 95% level of confidence to determine the differences between treatments.

RESULTS AND DISCUSSION

This research results showed that biochar had significant influence on mortality of *P. coffeae* nematodes. Laboratory study showed that biochar with concentration 4.0% killed 74.50% *P. coffeae* nematodes. Concentration of 0.5% of biochar only killed 37.5% of the nematodes or the lowest percentage (Table 1). Several studies on effectiveness of biochar to control plant disease had been conducted previously. Active charcoal was able to control stem cancer of nutmeg by absorbing and binding the liquid produced by the plants from the wound caused by the disease (Anonymous, 2010). The liquid seeped through the wound caused by the cancer would enter or be bound in active hollow of biochar absorbing the liquid. The zig-zag active biochar hollow made it easier to absorb the liquid. Biochar was also effective to reduce root decaying disease due to *Fusarium virguliforme* (Rogovska *et al.*, 2014).

In the study, the effectiveness of biochar to eradicate *P. coffeae* nematodes was similar to that of chemical nematicides. Three percent of biochar resulted in 62.50% mortality rate

Table 1. Effect of biochar on mortality of *P. coffeae*, 24 hours after treatment

Treatment	Mortality, %*)	Efficacy level, %**)
Biochar 4.0% (A)	74.50 a	74.50
Biochar 3.0% (B)	62.50 ab	62.50
Biochar 2.0% (C)	50.50 ab	50.50
Biochar 1.0% (D)	50.00 ab	50.00
Biochar 0.5% (E)	37.5 b	37.5
Control 0 (F)	c 0	

Notes: *) Values within the same column followed by the same letter (s) are not different according to Duncan's Multiple Range Test at 5% level); **) Calculation of effication level based on Schneider-Orelli's formula (Ciba – Geigy, 1981).

with 62.50% efficacy; moreover, 4.0% biochar killed *P. coffeae* nematodes with more than 70% level of efficacy. The findings of a study with the same method showed that 1 g/L of carbofuran killed 67.5% of nematodes with the efficacy level of 61.6% (Wiryadiputra, 2010).

The in vivo study showed that 4.0% biochar was the most effective dosage to reduce the *P. coffeae* parasitic nematodes in coffee (Figure 1). There were approximately 118 *P. coffeae* paracitic nematodes in 4.0% biochar, while population of the organism in the control was 771. It was predicted that biochar decomposition in the soil produced toxic compound that reduced the population of the *P. coffeae* parasitic nematodes. Wiryadiputra (1995) stated that adding organic substance and rice husk ash on coffee seedlings suppressed the parasitic nematode populations to approximately 70%. It was related to the existence of agents that became the natural enemies of nematodes in organic substance. Toxic compounds for parasitic nematodes were produced from the decomposition of the organic materials and the effects of the growth of plants so that the plants became tolerant. Organic substance could create favorable conditions for root to grow which further accelerated the growth of roots and increased the use of soil nutrient. In other words, organic substance enhanced plant tolerance against damage caused by nematode.

Based on DMRT analysis, it was found that 0-4% biochar influenced on population of *P. coffeae* in the roots of Arabica coffee seedlings. The higher biochar concentration was, the lower population of the *P. coffeae* nematodes was. Yet, there was one treatment, 2.0% biochar, where the average population of *P. coffeae* nematodes was higher than the 1.0% and 0.5% nematodes. It was predicted that eventhough the biochar was not applied, the *P. coffeae* nematodes would insert into the root tissue. According to Jones *et al.* (2013), *P. coffea* attacks epidermis, endodermis and cortex of roots, particularly young roots. The observation revealed that the population of *P. coffeae* in the soil was fewer than that in the roots. The population of *P. coffeae* in the soil in the treatments of 0%, 0.5%, 1.0%, 2.0% and 4.0% were 0%, 112.5%, 112.5%, 168.75% 0% and 37.5%, respectively (Figure 1). The average population of *P. coffeae* in each of the treatments were not consistent. It happened probably since most of the *P. coffeae* nematodes had entered Arabica coffee roots so that their population in the soil was fewer than that in the roots.

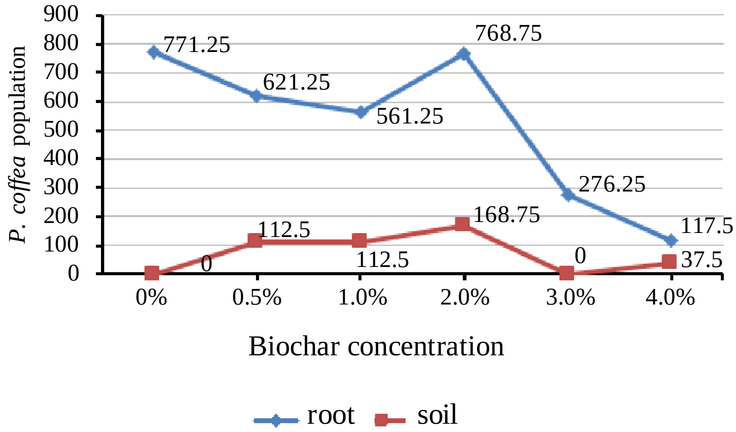

Figure 1. Effect of biochar on *P. coffeae* population in Arabica coffee seedlings

The percentage of root damage in 0.5%-4.0% biochar was between 55% and 76.3% (Figure 2). Based on the findings, biochar did not have any influence in the percentage of root damage (lroot lesion). It was due to microorganism in the soil such as bacteria and fungi. According to Jones *et al.* (2013), *Pratylenchus* infestation result in root damage which eventually decrease plant growth, increase water stress susceptibility and develop brownish root lesion. Decreased root growth is followed by decomposed root caused by secondary attack by fungi and bacteria. It generally would also be followed by flea infection (*Planococcus* spp.) covered by a type of fungus called *Diacanthodes* spp. (Pfaltzer & Fluiter *cit*. Wiryadiputra, 1995). In plantation, nematode infection was worsened by secondary infection from other pathogens; wilt disease caused by *Rhisoctonia solanacearum* and *Fusarium oxysporum* increased because of *Meloidogyne incognita* nematodes (Carneiro *et al*., 2010).

Root demage caused by nematode infection had direct influence towards the growth of plants. The plants would not grow well since the damaged roots could not work as it should. Some effects of nematode infection were stunted plant growth (stunted, wilt, even death) and decreasing yield (Dropkin, 1991). Pradana (2014) stated that the damage on the roots was affected by population of nematodes in the soil around the plants. Brooks (2004) had similar idea that the percentage of damage in banana roots had positive correlation to the population of *Pratylenchus goodeyi* nematodes. The higher population of *P. goodeyi* nematodes was, the more damage found on the banana roots.

Based on DMRT analysis, biochar significantly influenced number of leaf and height of the plants. During the testing on the glass house, the number of leaf and the height of the coffee seedling in the 2.0% biochar treatment were higher than those in other treatments (Figure 3). The findings of similar studies also revealed that biochar significanly influenced plant height and number of leaves of 40, 60 and 80-days mahagony (Helmi, 2014). Lehmann & Rondon (2006) mentioned that biochar may improve the growth and available nutrition in cowpeas (*Vigna unguiculata*) and rice (*Oryza sativa* L.) although the N concentration on the leaves decreased and the absorption of P, K, Ca, Zn and Cu by the plants increased.

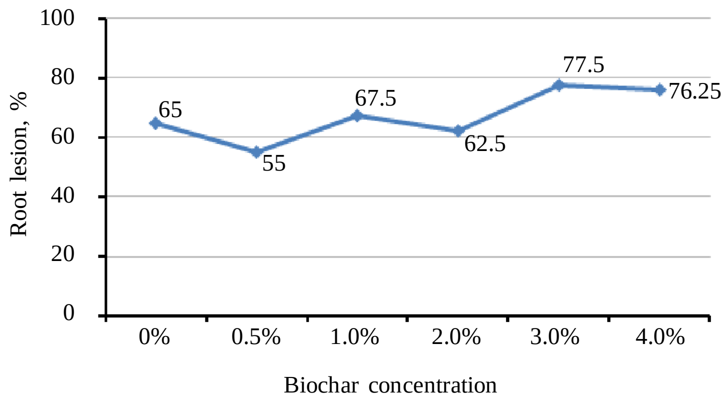

Figure 2. Effect of biochar on root lession caused by *P. coffeae* of Arabica coffee seedlings

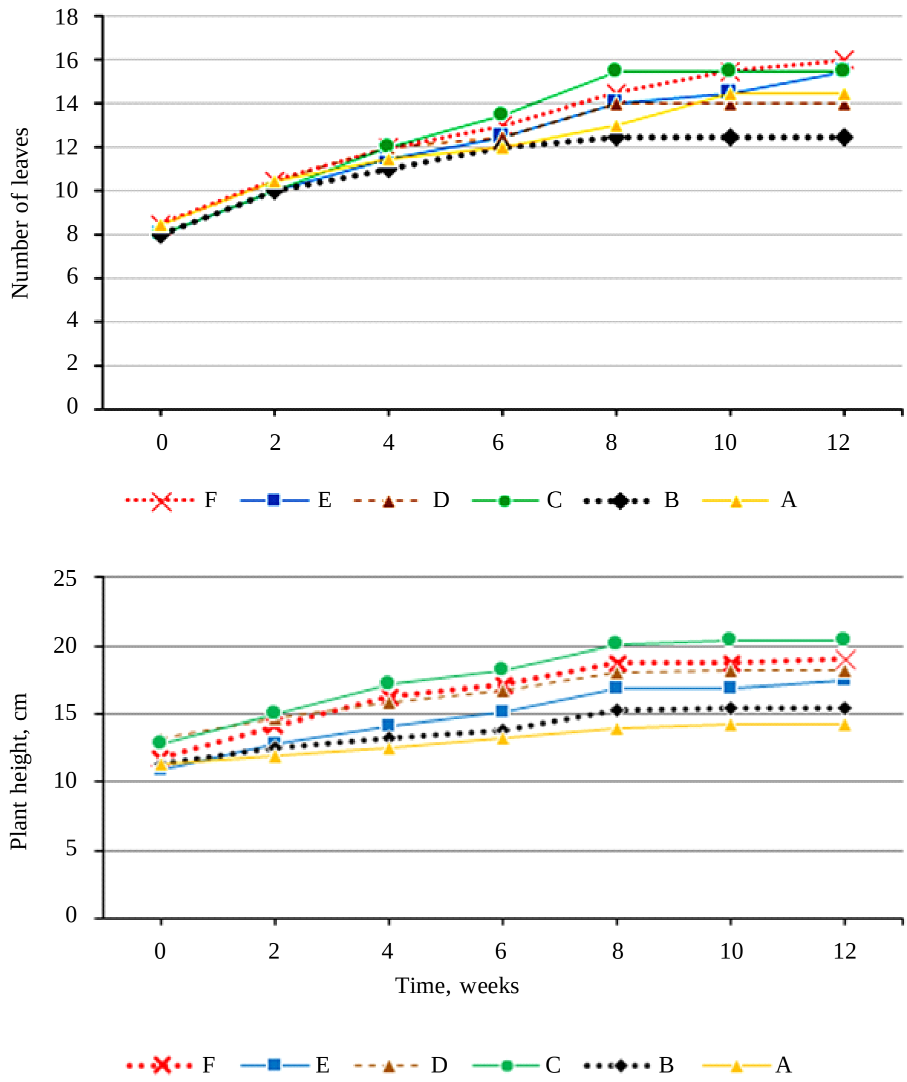

Figure 3. Effect of biochar on number of leaves (A) and height (B) of Arabica coffee seedling
Notes: (Explanation of treatment codes see Table 1)

Table 2 showed that 2.0% biochar treatment resulted in higher weight of fresh roots, fresh and dry shoots than the 3.0%, 1.0% and 0.5% biochar treatment, but not significantly different from the control. Nonetheless, there was a tendency that the 2.0% biochar treatment resulted in higher fresh roots, fresh and dry shoots weight than the control. The findings of Carter *et al*. (2013) indicated that applying 50-150 g/kg of biochar promoted growth and biomass of mustard (*Brassica chinensis*). It was related to increasing cation content (K, Ca, and Mg) and pH in the soil. The high nutrient available for the plants is the result of increased nutrients directly from the biochar and increasing nutrient retention (Lehmann *et al*., 2003). Beside that, biochar also increased the reach of plant roots making it easier for plants to get nutrients and water (Dou *et al*., 2012).

Table 2. Effect of biochar on fresh weight of roots and shoot, and shoot dry weight of Arabica coffee seedlings

Treatment	Fresh weight, g		Dry weight of shoot, g
	Roots	Shoot	
Biochar 4.0%	0.59 b	1.93 c	0.60 c
Biochar 3.0%	1.02 ab	2.58 bc	0.80 bc
Biochar 2.0%	1.31 a	4.60 a	1.44 a
Biochar 1.0%	1.28 a	3.54 ab	1.11 ab
Biochar 0.5%	1.10 ab	3.33 abc	1.09 ab
Control	1.14 ab	3.88 ab	1.19 ab

Notes: Values within the same column followed by the same letter(s) are not different according to Duncan's Multiple Range Test at 5 % level.

Basically, all organic substances applied to the soil improved various functions of the soil such as retention from various essential nutrients for the growth of plants. Lehmann (2007) mentioned that biochar was more effective to maintain the amount of nutrients in the soil compared to other organic substance such as leaves, compost or manure. Biochar also sustained that could not be retained by other organic substance for the soil. As addition, biochar provided suitable growing media and elevated activities of different soil biota. It increased the level of growing susbtance that along with the nutrients in the soil simultaneously improved the growth and production of plants (Pujiyanto, 2013).

CONCLUSION

Biochar application at concentration range of 3-4% in laboratory experiment controlled *P. coffeae* at level of effectiveness 62.5-74.5%. Application of biochar at concentration range of 0.5-4% could control *P. coffeae* at seedling stage. Application of biochar 2% produced higher fresh weight of root and shoot, and dry weight of shoot larger than control.

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