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RESEARCH PAPER

Termites and *Rhizoctonia* sp.: major problems in robusta coffee cuttings cultivated on different planting media

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ABSTRACT

Robusta coffee is one of the plantation commodities vegetative propagated through cuttings to keep them in the same characteristics as their parents. The main problems in cuttings propagation are pests and diseases are cause damage to the planted cuttings. This research aimed to study the causal agent and its damage to robusta coffee cuttings planted on different media. The research was conducted from September 2020 to March 2021 in the experimental field station (KP) Pakuwon and the Integrated Laboratory of Balittri. The arrangement of the experimental design was a split plot with two repetitions. The main plot was the type of media planted consisting of sand, sand + fertilizer (1: 1), sand + soil (1: 1), soil + fertilizer (1: 1), sand + soil + fertilizer (1: 1: 1). The subplot was a Robusta coffee clone consisting of BP308, BP393, and SA203. The results showed that the highest percentage of damage caused by termites and *Rhizoctonia* sp. was observed on the cuttings planted on sand + fertilizer mixture media while the lowest was on sand media. The termites found in the robusta coffee cuttings were *Macrotermes gilvus*.

Key words: clone, damage, fungi, insect, propagation

INTRODUCTION

Coffee (*Coffea canephora* L.), especially robusta coffee, is one of the important estate crops cultivated in Indonesia. The data from the Directorate-General of Estate Crops of Indonesia mentioned that 68% of the coffee plantation in Indonesia is robusta coffee covering 860.094 ha in 2018 and 862.049 ha in 2019 (Dirjenbun, 2019). Their potential to grow optimally in the lowland and specific taste makes robusta coffee popular as farmers' favorite variety. National production of robusta coffee in 2019 was 531.558 tons with a production average of 789 kg/ha (Dirjenbun, 2019). The high production of robusta coffee is affected by the success of coffee propagation in the nursery.

The propagation of robusta coffee was performed using the vegetative technique of cuttings. The cuttings method has been reported to have some benefits such as guaranteeing the fruit quality produced by its offspring, uniform offspring, and expressing the same character as the parents (Prastowo et al., 2010).

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Propagation of robusta coffee through cuttings in the nursery area experiences various problems, pests, and diseases that cause severe damage to most of the cuttings. The occurrence of pests and diseases in the nursery is mainly affected by the suitable environment and field activities that support pest and disease transmission. Among those factors, the media is important for the development and transmission of pests and diseases. The physical structure of the planting media can cause high humidity that affects the activity of pathogens and insects. The research objective was to study the causal agent and its damage to cuttings of 3 clones of robusta coffee planted on different media.

MATERIALS AND METHODS

Research Site. The research was conducted in coffee nurseries location of Experimental Field Station (KP) Pakuwon (6049'19.5"S 106044'20.7"E) Indonesian Industrial and Beverages Crops Research Institute (Balittri), Sukabumi.

Experimental Design. The research was designed in a split plot with two replications. The main plot was the treatment of planting media composition that consisted of sand (Sd), Sand + fertilizer (1:1) (SdF), Sand + soil (1:1) (SdS), Soil + fertilizer (1:1) (SF), sand + soil + fertilizer (1:1:1) (SdSF). While the subplot was robusta

coffee clones that were commonly used by the coffee farmers, consisting of BP308, BP939, and SA203. The observation was performed by regular visual observation for three months at seven days intervals. The total observed samples were 46 cuttings of each replication, so the total sample overall is 1380 cuttings.

Planting Media Preparation. Planting material was a an orthotropic branch or scion (wiwill buds) with upward branch growth. The scion was harvested from several clones of coffee BP 939, SA 203, and BP 308 that grow in the scion field of Pakuwon Experimental Field, Balittri. The selected cuttings material was two segments selected from two upper and lower segments (segments 1 to 2 and 3 to 4). Another required material for planting media cuttings consisted of sand, topsoil, and fine chicken manure. The composition used was sand, soil, and chicken manure according to treatment in percentage volume (v/v). Before mixing, the air-dried media was sifted using a sieve measuring 35 messes. Then, the sifted media was mixed according to the treatment and stirred evenly to obtain the homogeney media. Next, the media placed into a seeding tank made of cement measuring 140 × 700 cm, and each different media was bordered to prevent mixing.

Planting and Cuttings Maintenance. Orthotropic scion of clones BP 939, SA203, and BP 308 used four segments collected from scion garden of Balittri. Segment number 1, 2, 3, and 4 were cut using cutting scissors. The base part of each cutting was slashed obliquely with one side longer than another (checklist shape). The cuttings were from orthotropic shoots in single-node cuttings. The leaves were cut up to ¾ part to keep the photosynthesis process for energy (carbohydrates) supply so the cuttings were able to form shoots and roots.

Before planting, the media was sprayed with 10 g of fungicides Dithane M-45 5% dissolved in 5 L of water. At the first week of cuttings planted, the media was left without irrigation to maintain applied growth hormones and maximize penetration that stimulates tissue in root growth initiation. Cuttings planted in the media according to the treatment.

The planting area was covered using a semi-permanent lid made of transparent or UV plastic after planting. Maintenance during planting including irrigation and fungicides application conducted once a week. The observation of pest and disease symptom was conducted from 3 days after planting (DAP) until 90 DAP with 3 days intervals. The number of damaged cuttings was recorded and removed from the planting area to reduce the pathogen infestation. The lid was kept closed and only opened for maintenance and observation.

Isolation of Pathogen in Coffee Cuttings. Isolation was carried out by collecting infected cuttings that showed blackening from stem to leaves. In the laboratory, infected cuttings were sterilized using alcohol and then rinsed using sterilized water. Sterilized cuttings were cut and then planted in the 2% water agar media then incubated at room temperature. Fungi that grew from the cutting were transferred to PDA and were then identified.

Pathogenicity. Koch's postulate was performed to confirm the fungal isolate. The plant samples were a scion of clones BP 538 that cut about 2–3 segments, washed in running water, then cleaned using sterile distilled water two times, and dried with sterilized tissue paper. The base of the cuttings was applied moistened cotton with distilled water to keep the scion from wilting during observation. Each fungus isolate was inoculated on 3 stems of coffee cuttings by the wound method using a sterilized needle. Inoculation was carried out by taking pieces of the fungus isolate place in the wound area (modified method of Ismail et al., 2012). Then the coffee cuttings were stored in a plastic container measuring 40 × 30 × 10 cm that was padded with sterile tissue moistened with distilled water. The disease development was observed every day until symptoms appeared. Reisolation was conducted to confirm the pathogen.

Identification of Pathogen Found on the Coffee Cuttings. The purified fungi then identified morphologic based on the shapes, color, and the colonies growing type. Then the long and wide hyphae, conidia, and body shape were measured (Waalwijk et al., 2011). The result was then compared with the fungi identification book i.e. Illustrated Genera of Imperfect Fungi (Barnett & Hunter, 1998).

Identification of Pests Observed on he Coffee Cuttings. Observation of pests on coffee cuttings was carried out simultaneously with observation of diseases. The presence of the pests in coffee cuttings is indicated by some symptoms such as the fed bark, dry and hollow stem. Invaded coffee cuttings was collected and carefully examined. Insect pests found in the occupied coffee cuttings were collected and placed in plastic bottles (5 × 15 cm) filled with 70% alcohol. Identification of the pests was conducted in

the laboratory using an identification book i.e. The Insect of Australia A textbook for students and research Workers (Commonwealth Scientific and Industrial Research Organization (CSIRO), 2000) and a termites identification book i.e. Termites of Thailand (Ahmad, 1965).

Data Analysis. The data was analyzed using ANOVA followed by Tukey Test 5% with Statistical Tool for Agricultural research (STAR) ver 2.0.1 (Azrai et al., 2017).

RESULTS AND DISCUSSION

Damage Caused by Pests and Diseases. The results showed that the planting medium affected the infection by pathogen, but termite infestation was affected by the clones (Table 1). The highest survival rate of each clone was not significantly different. The study by Muliasari & Nurhikmah (2019) showed that BP 308, BP 913, BP 436, SA 237, BP 538, and BP 936 are resistant to the pathogen. Anderson et al. (2017) stated that several coffee clones can show similar resistance to the diseases. However, Hulupi et al. (2012) stated that each clone has a different reaction to the pathogen infestation. Some are showing resistance and other clones are susceptible.

The highest damage caused by pathogen infection was observed in fertilizer + sand media, followed by fertilizer + soil media, sand + soil, fertilizer + sand + soil, and the lowest was found in sand media (Table 2). The damage was affected by the difference in the structure and material of each planting medium. Fertilizer + sand media has material, structure and substance that can support the growth of the pathogen.

Planting media serves to maintain the humidity of the rooting roots, provide sufficient air, and maintain the availability of nutrients (Sumartini, 2012). Among all planting media used in the treatment, sand was a medium that has high porosity. This causes the sand to have a low ability to hold water. These conditions make the fungal pathogen spore difficult or even failed to emerge and develop in this medium. In addition, the sand media has lower organic matter than other media which caused the disease development relatively slow or inhibited. The soil-borne pathogen is mostly found in soil containing high organic matter, pH around 3.5–6, and soil temperature around 25–32 ºC (Milati & Nuryanto, 2019; Sumartini, 2012).

Termite invasion on coffee cuttings was not affected by differences in planting media. The damage caused by termites was very low, only reaching 1%

Table 1. Analysis of variance of the damaged robusta coffee cuttings caused by termites and *Rhizoctonia* sp. planted in different planting media and clones

Source	Percentage of Damaged Plant			
	Termite	P Value	*Rhizoctonia* sp.	P Value
Clone	ns	0.82	ns	0.09
Media	ns	0.47	**	0.02*
Clone*Media	ns	0.47	ns	0.33

Note: * and ** significant at the 5% and 1% levels respectively; ns= not significant.

Table 2. Statistical comparison of damage percentage of robusta coffee cuttings planted in different planting media caused by termites and *Rhizoctonia* sp.

Treatment	Percentage of Damaged Plant (%)	
	Termites	*Rhizoctonia* sp.
Sand fertilizer (SdF)	0.00	56.52 a
Soil fertilizer (SF)	0.72	39.85 ab
Soil sand (SSd)	1.08	23.55 bc
Soil sand fertilizer (SSdF)	0.36	21.38 bc
Sand (Sd)	0.00	8.70 c
CV (%)	273.97	70.79

Note: Values in the same column with different letters shows significant difference according Tukey Test at 5% error level; CV= coefficient of variation

(Table 2). Sand media tends to be dry and contain low organic matter causes reducing in termite infestation (Arif et al., 2019). Even though the damage and population of the termites were relatively low, however, the presence of termites in this cutting need to be carefully monitored. In some cases, termites are very destructive pest which can cause damage up to 80%, especially in heavy infestation (Ambele et al., 2018).

Pathogen Found in Coffee Cuttings. There were nine fungal isolates which were suspected as plant pathogens, were successfully isolated from the symptom and suspected as plant pathogens. The Postulate Koch test revealed that only one isolate (code A) expressed similar symptom as observed in the field (Figure 1). This result confirmed that among nine fungal isolates, only the fungi with code A were a pathogen. The symptoms was easily recognized by blackening on the cuttings stem that extend to all parts of the cuttings stem. The white hyphae of the pathogen emerged in the symptomatic area (Figure 2). Further infection caused the dead of cuttings.

Fungi code A has a characteristic colony light brownish-white with a hilly surface like cotton, with diameter colony was 9 cm filled the petri dish on day 8 (Figure 3). The mycelium branching was elbow-shaped (Figure 4). Based on the morphological observations revealed that the fungal pathogen infected the cuttings was in the genus of *Rhizoctonia* (Novina et al., 2012). Soelistijono et al. (2011) reported that the colony of *Rhizoctonia* spp. was white and browny. It has been reported that *Rhizoctonia* spp. that isolated from the same plant will express the different colors (Soelistijono et al., 2011). The mycelium of *Rhizoctonia* sp. was elips connected with browny thread. The specific character of this fungi was elbow-shape of the braches and mostly found in the soil. While the sclerotia were irregular shapes and brown (Dwiyanto et al., 2017). The hypha of *Rhizoctonia* sp. can detect the opened stoma of the host around, so the hypha development was heading to the opened stoma to enter through the gap or hole on it (Basu et al., 2016).

Rhizoctonia sp. is one of the pathogens with various host plants, such as Paddy (*Oryza sativa* L.), corn (*Zea mays* L.), barley (*Hordeum vulgare* L.), sorghum (*Sorghum vulgare* Pes.), potato (*Solanum tuberosum* L.), soybean (*Glycine max*), groundnut (*Arachis hypogaea* L.), cabbage (*Brassica oleracea*), lettuce (*Lactuca sativa* L.), stevia (*Stevia rebaudiana*), weed, and coffee. The infection of this fungus on the leaves was recognized as leaf blight (Yang & Li, 2012). This fungus was also reported found in the seeds (Ajayi-Oyetunde & Bradley, 2017).

Generally, *Rhizoctonia* sp. consisted of three groups based on the number of the nucleus, which was: uninucleate, binucleate, and multinucleate (Otero et al., 2002, Yang & Li, 2012). The group of binucleate *Rhizoctonia* has around 1-3 nucleus in one cell, while in multinucleate has more than 3 nucleus. The binucleate *Rhizoctonia* commonly found has mutualism symbiosis with orchids as endophytic fungi that provide nutrition for orchids (Dwiyanto et al., 2017). While the multinucleate *Rhizoctonia* were common as pathogen of the plant (Otero et al., 2002).

Pest Observed in Coffee Cuttings. Termites were only pest found in the cuttings causing thefed bark and hollow (Figure 4A). The cuttings became dried-

Figure 1. Cuttings that have been inoculated with fungal isolates found in cutting seedlings. A. Asymptomatic cuttings; B. Symptomatic cuttings.

Figure 2. The symptoms on the part of cuttings stem. A. Healthy cuttings; B. An infected cuttings.

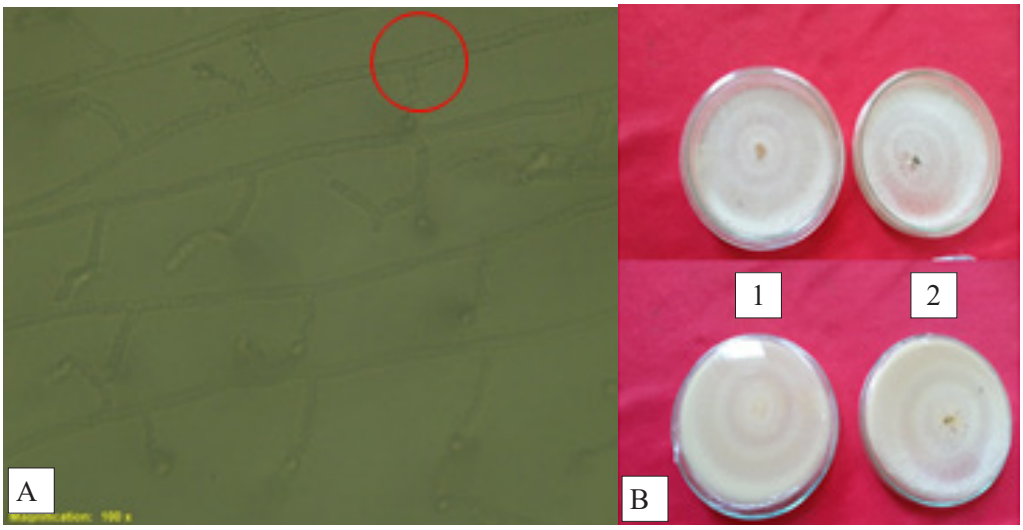

Figure 3. The fungus of *Rhizoctonia* sp. A. Microscopic view of fungi morphology; B. Colonies on PDA media; (1) The isolates growth from infected cuttings; (2) The results of the Koch Postulate test.

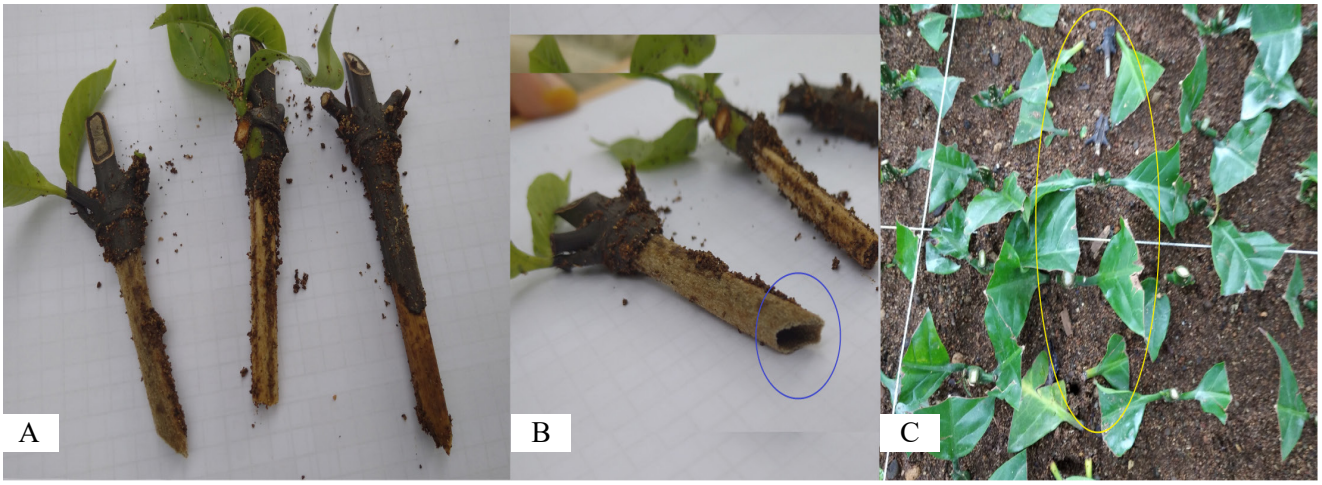

Figure 4. The termite infestation. A. Symptoms on cuttings; B. Coffee stems are hollow/perforated and brittle; C. Infested cuttings were located adjacent to each other.

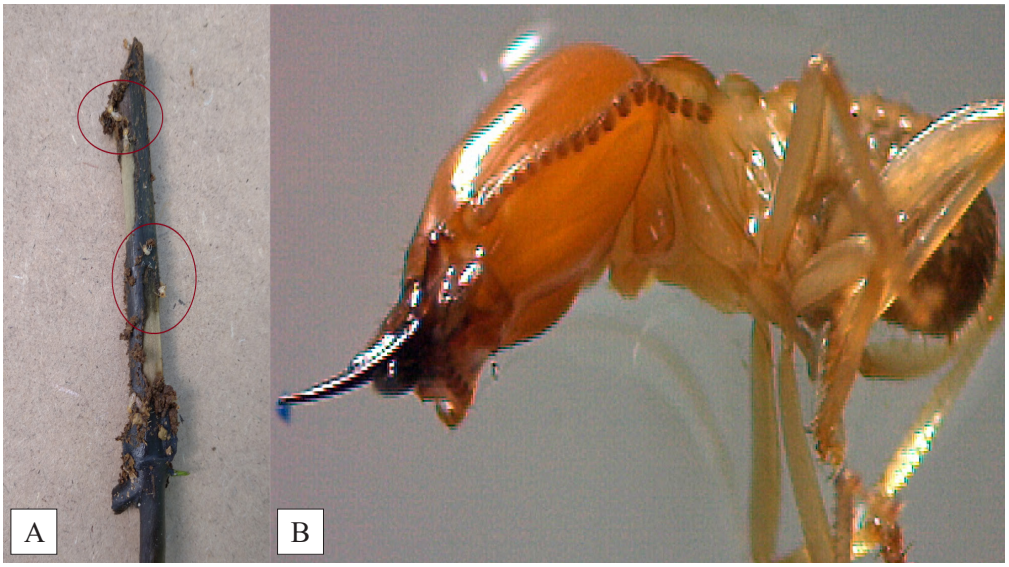

Figure 5. The termites. A. Termites on coffee stem; B. The morphology of discovered termites.

hollowed because the inner part was hollowed out by termites (Figure 4B). In the nursery, the cuttings damaged caused by termites were usually adjacent to each other (Figure 4C). It showed that the termite's activities in the ground have caused damage or even death of the cuttings.

The termites (soldiers) collected from the field has 0.6 cm in size and have antennae with 17 segments. The morphological characters showed that the termites were in the Family Termitidae, genus Macrotermes. Further identification showed that the termite's species was *Macrotermes gilvus* (Figure 5). Termites (*Macrotermes* spp.) are known as a pest on coffee nurseries in Ghana. The termite invasion was from the root to the stem (UCDA, 2019). The high damage caused by the presence and total of organic matter in the area. Irregular irrigation also caused the media to be dry enough for termites to survive and develop in the area. According to the situation, one recommendation to control the population of termites is regular irrigation, and sanitation of the media including removing falling leaves and twigs from the planting media (UCDA, 2019). Termites consume the twigs and branches for food using microorganisms inside their digestive system (Grace, 2014). Installation of a cover frame made from light steel can be used as an alternative to reduce the termite's infestation in the nursery (Paul et al., 2018).

CONCLUSIONS

The lowest damage percentage was coffee cuttings planted in sand media, while the highest was in media combination of sand and fertilizer. The symptom of the disease was blackish rot and plant died that showed characters of *Rhizoctonia* sp. The damage symptom of *Macrotermes gilvus* infestation was hollowed/perforated cuttinga, brittle and truncated until died.

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AUTHORS' CONTRIBUTIONS

S, MP, NKF and DP designed the experiment. SS, MP and NKF conducted the experiments, SS and MP analyzed the data, and wrote the manuscript as well as provided the basic data of the research. NKF and DP helped revised the manuscript. All authors reviewed and revised the manuscript.

COMPETING INTEREST

The authors declare that there was no conflicts of interest in the research activity and writing process.

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