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Earthworms as a vector of *aspergillus niger* dispersal to enhance the decomposition of oil palm trunks

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Abstract

Effective plant residue management is crucial in the replanting operation of mature oil palm plantations. Control of the large amount and volume of the fallen palm trunks is challenging. Biological agents such as earthworms and fungus could enhance organic matter decomposition. Thus, this study aimed to explore the dispersal of cellulose decomposing fungi with the help of earthworms. This study examined the role of earthworm species (endogeic and anecic) on dispersing Aspergillus niger from one oil palm trunk to another. To study this effect, an open container was divided into two compartments (A and B). Earthworms and the inoculated oil palm trunk pieces with A. niger were applied at compartment A that consisted of sterile soil. On the other hand, compartment B was filled with sterile soil and sterile pieces of oil palm trunk. The compartment barrier between A and B was then opened for 40 days. Earthworm treatments for A included control (without earthworm), four anecic, eight anecic, two anecic+two endogeic, four endogeic, eight endogeic, four anecic+four endogeic earthworms. Measurements included percentage of earthworms that moved from compartment A to B, the presence of A. niger in earthworms' intestine and on the oil, palm pieces in B, and C and N concentration of palm pieces. The results showed that endogeic worms had higher movement compared to anecic worms. Earthworms moved from the denser population site to the less populated one. The species and abundance of earthworms had a significant effect on the presence of A.niger in the worms' intestine and on oil palm pieces in compartment B. In particular, anecic worms induced the highest A.niger population. Nevertheless, the decomposition of oil palm pieces was mostly controlled by the abundance of earthworms. Carbon of palm trunk was reduced by 20% in the treatment with the largest population of earthworms. This study demonstrated that the earthworms could be used to enhance the decomposition of oil palm trunk pieces and disperse beneficial fungi.

Keywords: Anecic, *aspergillus niger*, endogeic, oil palm, vector, organic matter decomposition

Introduction

Oil palm plantations are a major contributor to the economy of Indonesia. Mature oil palms need to be rejuvenated to ensure cost-effective production. Effective plant residue management is the most essential steps in the rejuvenation and replanting of mature plantation crops. In oil palm plantations, unproductive mature trees need to be uprooted and cleared so that new trees can be replanted. In the replanting operation, mature palms were uprooted, and their trunks were cut and chipped into smaller pieces and spread on the soil of the plantation. One hectare (ha) of oil palm plantation can produce around 140-144 palm trunks. This unused biomass can be returned to the soil as organic carbon (Minasny *et al.*, 2020). However, oil palm trunk has a high cellulose content (32.55%), and it decomposes slowly (Taniwiryono, 2014). The large amount and volume of the palm trunks can be a nuisance and disturb the replanting process. Most plantation companies chipped oil palm trunks using heavy machinery. Even though the size of the palm trunk has been minimized, it can still attract the development of *Oryctes rhinoceros* L. pest (Chung, 2012). To avoid the explosion of the population of pests, such as *O. rhinoceros*, it is necessary to make sure the trunk pieces decompose rapidly and do not become a host for pests. The application of chemical decomposer agents on oil palm trunks has been suggested to accelerate decomposition, however, it is still not feasible for large scale plantations because it is labor intensive and expensive.

Researchers are now exploring the use of biological agents such as earthworms and fungus to enhance organic matter decomposition (Medina-Sauza *et al.*, 2019). Earthworms are well known as carriers of microorganisms, including actinomycetes. Spores attached to their bodies and castings could significantly spread actinomycetes in an area

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(Jayasinghe and Parkinson, 2009). The feeding behavior of earthworm, which is the ingestion of soil and litter, can ultimately host beneficial microorganisms in their castings (Eisenhauer *et al*., 2012; Ali *et al*., 2015). Microbes are responsible for the decomposition and turnover of nutrients and carbon in soils (Byzov *et al*., 2015; Arai *et al*., 2017; Xue *et al*., 2018). The presence of beneficial microbes can improve soil health. Earthworms could also control the presence of harmful microbes in their casting through polysaccharides and coelomic fluid. Earthworms produce polysaccharides and have broad-spectrum antibacterial activities against pathogenic bacteria and plant pathogenic fungi, as found *in vitro* (Wang *et al*., 2007). The coelomic fluid of earthworm species *Eudrilus eugeniae*, contains compounds with pesticidal, insecticidal, antifungal and plant hormone (derivative, indole-3-acetyl-L-valine) properties (Nadana *et al*, 2020).

In particular, *Aspergillus niger* is a fungus that can synthesize cellulase enzymes, and therefore can be used as a decomposing agent for cellulose-rich oil palm trunk. *Aspergillus niger* is used in the cellulase enzyme industry (Mrudula and Murugamal, 2011). *Aspergillus niger* strains used by Din *et al*. (2019) excrete extracellular phosphate solubilizing enzyme such as phytase (133 UI in 48 h of fermentation) and phosphatase (170 UI in 48 h of fermentation) which can solubilize the rock phosphate and make it available to plants. These excellent characteristics can be tapped to not only enhance decomposition but also increase the efficiency of phosphorous uptake by oil palm.

Thus, this study aims to utilize earthworms to disperse *A. niger* in oil palm plantation to enhance the decomposition of palm trunks. This study was conducted an experiment to investigate the most effective earthworm species and its abundance as a vector for dispersing *A. niger* on oil palm trunk pieces.

Materials and Methods

This research was conducted from February until July 2017 at the Laboratory of Ecology and Soil Sciences Faculty of Agriculture, University of Sumatera Utara, Medan, Indonesia.

Anecic (*Lumbricus terrestris*) and endogeic (*Pontoscolex corethrurus*) earthworms were selected as agents. Earthworms and pieces of palm trunk were taken from the oil palm plantation PTPN IV Marjandi estate in North Sumatra, Indonesia. *Aspergillus niger* was from a collection of Ecology and Biology laboratory of the Agriculture Faculty, University of Sumatera Utara.

The experiment was designed using a container made from an open-top wooden box (dimension 30 x 20 x 20 cm) without any hole at the bottom and sides of the box, as shown in Figure 1. The box was divided into two compartments: A and B, separated by a moveable sheet made from zinc. Both containers were filled with trunk pieces, and only compartment A received worms. Then the two compartments were opened to allow movements of earthworms and Aspergillus *niger* from A to B.

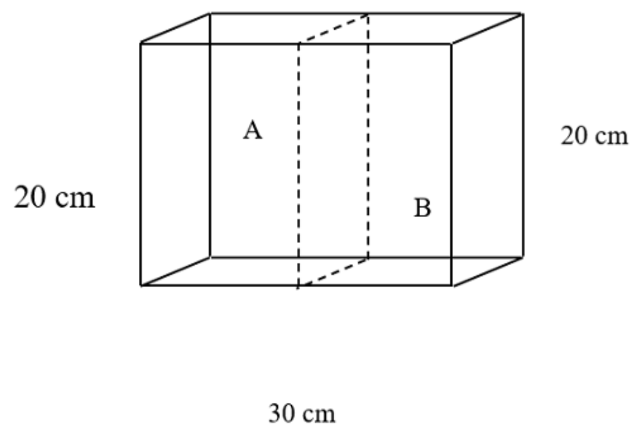

Figure 1: The experimental container, a wooden box separated into 2 compartments: A and B with a moveable sheet

The experimental design was a randomized block design consisted of 7 treatments of 2 species of worms and 3 levels of abundance, namely:

1. control (without earthworm),
2. four anecic earthworms,
3. eight anecic earthworms,
4. two anecic earthworms + two endogeic earthworms,
5. four endogeic earthworms,
6. eight endogeic earthworms, and
7. four anecic earthworms + four endogeic earthworms.

All treatments had 4 replications.

Preparation materials

Anecic and endogeic earthworms were collected from PTPN IV Marjandi oil palm plantation. The separation of endogeic and anecic worms was done manually by referring to the manual of the identification of earthworms (Blakemore, 2002). All worms obtained were soaked in sterile water to remove other materials and microbes in their intestines before being applied in the experiment.

Soil from the plantation was sterilized using electric soil sterilization, then 2.5 kg sterile soil was filled into both compartments of the experimental box. Pieces of oil palm trunk were chopped into 0.96 cm^3 in size and sterilized using autoclave. The C/N ratio of oil palm trunk was 33.97 (46.21% C and 1.36% N).

Implementation

An 80 g sterile oil palm trunks were immersed in the liquid cultures of *A. niger*, subsequently placed on the soil at compartment A. Similarly, another 80 g of sterile palm pieces were placed on the sterile soil at compartment B.

The earthworms were introduced to compartment A according to their prescribed treatments. Soil moisture in the experimental box was maintained at field capacity by adding sterile distilled water. After 40 days of earthworm's culture in compartment A or until the casting found near palm trunk pieces (Figure 2), the sheet barrier was removed to allow movement.

Figure 2: Casting near oil palm trunk pieces

Twenty-one days after the two compartments were connected, worms' observations were conducted. The parameters observed included the percentage of the species and the number of earthworms that had moved to compartment B. The CFU of *A.niger* in the earthworm's intestine was measured. In addition, the decomposition of oil palm pieces was measured via organic carbon, nitrogen content, and C/N ratio.

Earthworms in the B compartment were cleaned using tap water followed by sterile water. Then, the worms were placed in Petri dishes filled by sterile water for several hours until the water became muddy. The water was examined for the population of *A.niger* in the earthworm's intestine. Dilutions were carried out to 10^{-3} for the source of the inoculum.

Organic C was analyzed using the Walkley and Black method, and total N content was analyzed using the Kjeldahl method. The C/N ratio was calculated as C/N = total organic carbon/total nitrogen.

Statistical analysis

The observations of each parameter were analyzed by Analysis of Variance (ANOVA) using SAS version 9.4, subsequent testing with Duncan multiple range test at 5% level.

Results and Discussion

Percentage of the earthworms moved to compartment B

The percentage of worms moved from compartment A to B was influenced by the species and abundance of worms (Table 1).

The highest percentage of movement can be found in the treatment of 4 endogeic worms; around 75% of the worms in compartment A moved to B (Table 1). The endogeic worms tended to have a greater percentage of movement compared to the anecic worms. The movement is also a function of abundance, as shown by the amount of movement of 75% and 65.63%, respectively, for 4 and 8 endogeic worms. While for anecic worms, the 4 worms'

Table 1: Percentage of the earthworm type and population moved to compartment B

Treatment	Percentage of moving worms	
	Type*	Population
	-------------- % ---------------	
Four Anecic Earthworms	100 A	18.75 c
Eight Anecic Earthworms	100 A	40.63 c
2 Anecic + 2 Endogeic	0 A + 100 E	31.25 c
Four Endogeic Earthworms	100 E	75.00 a
Eight Endogeic Earthworms	100 E	65.63 b
4 Anecic + 4 Endogeic	18.19 A + 81.81 E	34.38 c

The numbers followed by the same letters in the same column means no significant difference by the 5% DMRT test; * notation for type: A is Anecic and E is Endogeic.

treatment only produced 18.75% movement and 40.63% movement for 8 anecic worms. According to Chatelain and Mathieu (2017), the dispersal behavior of epicic earthworms depends on the habitat quality and population density, but the responses also vary among species and behavior of endogeic earthworms.

The movement of worms in the mixed anecic and endogenic worms' treatment was dominated by endogeic worms, which shows that all of them moved (100%). The lowest movement percentage was found in the treatment of 4 anecic worms (18.75%). No statistical difference was found for the combination of 2 anecic + 2 endogeic worms, eight anecic worms, and 4 anecic + 4 endogeic worms. The nature of endogeic worms tend to move laterally below the soil surface, making them more likely to move to other sites compared to anecic earthworms, which tend to move vertically. Narayan *et al.* (2016) found that *P. corethrurus* has high consuming adaptability, which helps it to spread colonies in various habitats as compared to anecic.

Population of A niger on oil palm trunk pieces and in earthworm's intestine

The population of A. niger was determined by isolating A. niger from oil palm trunk pieces and from earthworm's intestine found in compartment B. The treatment effect of earthworm species and abundance significantly influenced A. niger population on palm trunk pieces and in the worm's intestine found in compartment B (Table 2).

The highest population of A. niger on oil palm trunk pieces in compartment B was found in the treatment 4 endogeic + 4 anecic worms, 132.5 x 103 CFU mL^{-1}, which was statistically different from all other treatments. Compartment B without earthworm has the least amount of A. niger (12.5 x 103 CFU mL^{-1}). Although there is no earthworm, A. niger can still spread their spores through the aerial transmission. However, the presence of earthworms significantly increased A.niger population on oil palm trunks in compartment B.

The earthworms interact with microorganisms in residues, although the interactions depended heavily on the earthworm species. Increased microbial numbers and activity have been reported during the vermicomposting process. This may be related to the passage of microorganisms present in the waste through the earthworm's gut. The casting adjacent to pieces of oil palm trunks in compartment B caused cellulolytic microbes to be found on the palm pieces. De Novais *et al.* (2019) demonstrated the trophic relationship between the earthworm P. corethrurus and three arbuscular mycorrhiza fungi species (Rhizophagus clarus, Claroideoglomus etunicatum, and Gigaspora margarita). Spore number, percentage of germinated spores, and most probable numbers of infective propagules of R. clarus and C. etunicatum were higher in earthworms than in soil-inocula, whereas the opposite trend was found with G. margarita, suggesting that the digestion of AMF propagated by P. corethrurus varied with the AMF species.

In the earthworm's intestine, the highest population of A.niger was found in the treatment with 8 anecic worms (230 x 103 CFU mL^{-1}), significantly higher than all other treatments. The lowest A. niger population in worm's intestine was found at 4 endogeic worms' treatment (22.5 x 103 CFU mL^{-1}).

The population of A.niger in anecic worm's intestine was higher than the endogeic. At the treatment where endogeic worms moved 65 - 75% to compartment B, but A. niger in the worm's intestine was only 22.50 - 158.75 x 103 CFU mL^{-1}. On the other hand, when the percentage of the anecic worms moved was only 18 - 40%, more A. niger could be found at 150 - 230 x 103 CFU mL^{-1}. Anecic earthworms consume organic matter, while the endogeic earthworms mostly ingest soil (geophagus). This diet preference causes anecic earthworms to eat more organic material from palm oil trunk pieces that already contained A. niger. Moreover, mucopolysaccharides are derived from microorganisms in the intestines of earthworms and the earthworms themselves create a favorable condition for microorganisms to grow, including A .niger.

The more worms moved to compartment B, the higher amount of A. niger could be found. In the treatment of 8 earthworms, there were 157 - 230 x 103 CFU mL-1 A. niger, while in the treatment of 4 earthworms, there were only 22 - 150 x 103 CFU mL^{-1}. Aspergillus niger, which was carried along with earthworm casts, spread into the soil or organic matter around the earthworm casts. Jayasinghe and Parkinson (2009) found that earthworm casts significantly caused an increase in the number of actinomycetes compared to natural soil. In comparison, the difference in the number of actinomycetes in worm species can be caused by the ability of worms to digest and accommodate microbial biomass, its ecological type, food characteristics, and the environment that supports the life of earthworms. Fungi, such as Penicillium, Mucor, and Aspergillus were found in the intestine of worms (Pizˇl and Nováková, 2003).

Organic C and N content of oil palm trunk pieces

Analysis of variance showed that the treatment of the type and number of earthworms affected the organic C content of the oil palm trunk pieces in compartment A. Various treatments did not significantly influence the organic C and N concentration of trunk pieces in compartment B (Table 3).

Table 2: The population of *A. niger* on oil palm trunk piece and on the worms intestine

Treatment	Population of *A. niger* in compartment B	
	on oil palm trunk piece	on the worm's intestine
	------------- x 10^3 CFU mL^{-1} -------------	
Without earthworms	12.50 d	-------
Four anecic earthworms	20.00 bcd	150.00 c
Eight anecic earthworms	60.00 b	230.00 a
2 anecic + 2 endogeic	32.50 bcd	28.75 d
Four endogeic earthworms	19.50 cd	22.50 d
Eight endogeic earthworms	52.50 bc	158.75 b
4 anecic + 4 endogeic	132.50 a	157.50 b

The numbers followed by the same letters in the same column means no significant difference according to the DMRT at α 5%.

Table 3: C and N content of oil palm trunk pieces in compartment A and compartment B

Treatment	Organic C	ΔC	Total N	ΔN
	--------------- %	---------------	------------- %	------------
Compartment A				
Without Earthworms	34.15	12.06 bc	1.59	-0.23
Four Anecic Earthworms	38.35	7.86 cd	0.93	0.43
Eight Anecic Earthworms	26.62	19.59 a	0.95	0.41
2 Anecic + 2 Endogeic	36.94	9.27 bcd	0.91	0.46
Four Endogeic Worms	38.98	7.24 d	1.16	0.20
Eight Endogeic Worms	25.99	20.22 a	1.25	0.12
4 Anecic + 4 Endogeic	33.80	12.41 b	0.95	0.42
Compartment B				
Without Earthworms	35.84	10.37	1.13	0.24
Four Anecic Earthworms	26.66	19.56	0.80	0.56
Eight Anecic Earthworms	26.97	19.24	0.70	0.66
2 Anecic + 2 Endogeic	29.29	16.92	1.00	0.36
Four Endogeic Worms	23.28	22.93	1.46	-0.10
Eight Endogeic Worms	32.39	13.82	1.20	0.16
4 Anecic + 4 Endogeic	33.76	12.45	1.13	0.24

Numbers followed by the same letter in the same column and same compartment means no significant different according to the 5% DMRT test; Δ C-organic is the difference between the initial C value of the study and the final C value. The initial C and N concentration of oil palm trunk pieces were 46.21% and 1.36%, respectively.

In compartment A, treatments of 8 endogeic worms and of 8 anecic worms showed the largest C change (ΔC = 20.22 and 19.52%, respectively), and significantly differed with ΔC of control (without earthworm). All other treatments did not show a significant difference from control. The results indicated that the treatment of 8 earthworms caused a larger reduction in C content of 12.41 - 20.22%. While the treatment of four earthworms only showed a C difference of 7.24 - 9.27%.

The alteration of organic C in compartment B was not affected by the treatment of species and populations of earthworms. However, most treatments showed an increased decomposition rate compared to control. The treatment of 4 endogeic worms caused the highest alteration in C values by 22.93%, followed by 4 anecic worms 19.56% and 8 anecic worms 19.24. This related to the percentage of earthworms that moved to compartment B on the treatment 4 endogeic (75%) was the highest and followed by treatment 8 endogeic (65.63%) (Table 1).

Earthworm is widely known to play an important role in nitrogen cycle in soil. Through their activities, they affect the mineralization of organic matter directly and indirectly. However, earthworm abundance and activities are also affected by organic matter. Desjardins *et al*. (2003) found that P. corethrurus was able to reduce 28% of the total carbon in plots inoculated with earthworms. P. corethrurus can dramatically affect the dynamics of soil organic matter.

Our research found that there was no relationship between the amount of nitrogen in oil palm trunk pieces and the presence of earthworm. The nitrogen content of the oil palm piece at compartment A ranged 0.93%-1.59%, while at compartment B ranged 0.70%-1.46%. Only two treatments with N content of oil palm trunk pieces higher than its initial N content (1.36%), they were the N content at the treatment without earthworm in compartment A (1.59%) and at the treatment 4 endogeic in compartment B (1.46%). During oil palm trunk pieces decomposition, the N content in the residues was fluctuating. The nitrogen content of oil palm trunk pieces at treatment using anecic type worm was the lowest. Maybe due to the fact that anecic earthworm is bigger than endogeic, hence the amount of carbon and nitrogen consume is more. It is also caused by the different feeding activity between endogeic and anecic earthworm. Among other 3 epi-anecic earthworm spesies , Lumbricus festivus have a higher contribution to surface leaf litter loss (Hoeffner *et al*., 2018). Two anecic ecological sub-categories have different impacts on soil functioning and each of them regroups earthworm species with similar behaviour (Hoeffner *et al*., 2019). They also found that FDAse activity was higher in earthworm burrows whereas acid phosphate activity was higher in earthworm middens.

Conclusions

This study showed that earthworms could be used to disperse beneficial fungi that can help enhance oil palm trunk decomposition. The percentage of the endogeic worm's movement was greater than that of the anecic worms. The higher the earthworms' population density, the higher the percentage of the earthworm movement. Anecic earthworm was a better A. niger vector than the endogeic worm species. Nevertheless, the decomposition of trunk pieces at this short-term observation is only affected by the earthworm abundance.

The results indicated that future work needs to verify the efficacy of introducing a mixture of earthworm species in the field.

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