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Exploration of *Pilobolus* sp. Fungi from Various Livestock Manures in Kedung Pacul Village, Klaten

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Abstract

Pilobolus is a coprophilous fungus belonging to the Zygomycota. *Pilobolus* sp. is called a coprophilous fungus because it can live in animal feces. The uniqueness of this fungus is that it can shoot its spores, so *Pilobolus* sp. is called a shotgun fungus. *Pilobolus* sp. exhibits a phototropism mechanism in which the sporangium shoots spores in the direction of light. *Pilobolus* has a unique living habitat, namely in the manure of herbivores such as cows, goats, sheep, etc. This habit of life may seem terrible to us, but fungi like *Pilobolus* sp. are very important in life because they are a type of decomposer, capable of breaking down organic matter from dead living things. This research aimed to explore the presence of the fungus *Pilobolus* sp. in various livestock feces in Kedung Pacul Village, Klaten. The method used was to cultivate the fungus *Pilobolus* sp. for seven days on various animal wastes placed in jam bottles. The results showed that the *Pilobolus* sp. fungus grew on various media of animal feces, such as horses, cows, goats, and pigs, with different growth times, and there were also spores shot on the glass walls that had been perforated.

Keywords: *Fungus, manure, Philobolus, shot-gun, spores*

Abstrak

Pilobolus adalah cendawan koprofil yang tergolong dalam Zygomycota. *Pilobolus* disebut cendawan koprofil karena dapat hidup di kotoran hewan. Keunikan dari cendawan ini adalah dapat menembakkan sporanya sehingga *Pilobolus* sp. disebut dengan shotgun fungi. *Pilobolus* sp. menunjukkan adanya mekanisme fototropisme dimana sporangiumnya menembakkan spora ke arah datangnya cahaya. *Pilobolus* memiliki habitat hidup yang unik, yaitu di kotoran ternak herbivora, seperti sapi, kambing, domba, dsb. Kebiasaan hidup ini mungkin tampak mengerikan bagi kita, tetapi cendawan seperti *Pilobolus* sp. sangat berperan dalam kehidupan, karena salah satu jenis dekomposer yang mampu memecah bahan organik dari makhluk hidup yang telah mati. Tujuan penelitian ini adalah untuk mengeksplorasi keberadaan jamur *Pilobolus* sp. pada berbagai feses hewan ternak di Desa Kedung Pacul Klaten. Metode yang digunakan yaitu menginkubasi cendawan *Pilobolus* sp. selama tujuh hari pada berbagai kotoran hewan yang ditempatkan pada botol jam. Hasil yang didapatkan bahwa cendawan *Pilobolus* tersebut tumbuh pada berbagai media kotoran hewan, seperti kuda, sapi, kambing dan babi dengan waktu tumbuh yang berbeda dan juga terdapat spora yang ditembakan pada dinding kaca yang telah dilubangi.

Kata kunci: *Cendawan, kotoran hewan, Pilobolus, shot-gun, spora*

INTRODUCTION

Fungi are a group of eukaryotic organisms with cells that have a nucleus membrane. The characteristics of fungi include being eukaryotic, having both unicellular and multicellular body forms, lacking chlorophyll, being heterotrophic, having a cell wall made of chitin, and being able to reproduce sexually and asexually (Hariana, 2005). Zygomycota is a type of fungi characterized by aseptate hyphae (lacking cross-walls) under normal or vegetative conditions. These fungi are coenocytic (having multiple nuclei in a single cell) and can form temporary dormant structures called zygospores. Most members of this class live on land, in soil, or on decaying plant and animal matter (Campbell et al., 2008). Coprophilous fungi are a group of fungi that grow on herbivore feces, which is a complex substrate containing digested plant remains, intestinal microorganisms, various additional components, and their nitrogen content. The pH and moisture levels of coprophilous fungal substrates are generally higher than most other substrates used by fungi

(Mumpuni et al., 2018). Coprophilous fungi have the ability and play a role in the process of animal feces decomposition (Babasaheb & Palghadmal, 2019). Fecal analysis in herbivorous animals has a positive impact on the digestive efficiency of other animals by providing a source of nutrition for certain arthropods in the fecal environment (Wicklow & Angel, 1974). The presence of fungi in feces is also a marker of environmental conditions because their diversity can indicate whether degradation is occurring in an ecosystem (Ebersohn & Eicker, 1992). Additionally, these fungi have potential as sources of antibiotics and enzymes, suggesting that they may be helpful for therapeutic use (Santiago et al., 2011).

Pilobolus sp. is an obligate coprophilous fungus that can be identified by its unique characteristics, including upright unbranched sporangiophores that grow towards light and sporangia that contain spores on top (Aluoch et al., 2015; Souza et al., 2017). *Pilobolus* sp. can be found in ruminant animal feces. Still, its presence has yet to be extensively studied in other herbivore feces or, for the first time, in omnivorous animals such as pigs. Therefore, this study aims to explore the presence of *Pilobolus* sp. in various livestock manure substrates.

MATERIALS AND METHODS

The method used in this study was purposive sampling. The samples were collected from the feces of horses, cows, buffaloes, pigs, rabbits, and goats, obtained from farms in Kedung Pacul Village, Gondangsari, Juwiring, Klaten. Fresh animal feces were moistened adequately and placed in transparent containers, with three replicates for each type of feces. The containers were loosely covered to allow for aeration, then wrapped with black manila paper, leaving one hole with a diameter of approximately 5 Mm on the side. The feces were incubated at room temperature. The growth of *Pilobolus* sp. was observed starting from the third day of incubation. The presence of black spots on the illuminated side of the container indicated the growth of the fungus. The fungal colonies were observed for morphological characteristics based on Keizer (2007), including bottle-shaped transparent reproductive structures, black sporangia, and transparent sporangiophores.

RESULTS AND DISCUSSION

Presence of *Pilobolus* sp. Fungi

Based on the obtained results, *Pilobolus* sp. fungi were found in four out of six used substrate samples (Table 1). *Pilobolus* sp. appeared in the horse, cow, buffalo, and pig feces samples on different days. The presence of the fungus was confirmed by the presence of black spots, which were spores, on the side walls of the containers used (Figure 1).

Table 1. Exploration Results of *Pilobolus* sp. from Various Substrates

No	Substrate	Emergence on day-
1	Horse feces	4
2	Cow feces	5
3	Buffalo feces	5
4	Pig feces	6
5	Goat feces	No growth observed
6	Rabbit feces	No growth observed

The fastest growth of *Pilobolus* sp. fungi was observed in horse feces after an incubation period of four days, followed by cow and buffalo feces after five days, and pig feces after six days. The difference in growth time is believed to be influenced by several factors. According to Babasaheb and Palghadmal (2019), factors that can affect coprophilous mycobiota include nutrient availability, ecological factors, pH, aeration, temperature, light preference, and predation. In this study, the different substrates are believed to be the primary factor causing the difference in growth time. Horses, cows, and buffaloes are herbivores, while pigs are omnivores, so the differences in their food sources will result in different compositions of their feces. The second factor is likely to be the incoming light. *Pilobolus* sp. fungi are light-sensitive, so the amount and quality of light received will affect their growth process.

Richardson (2000) stated that *Pilobolus* sp. is a coprophilous fungus found in various substrates, including deer, cow, rabbit, sheep, and grouse feces. However, in this study, *Pilobolus* growth was not observed in goat and rabbit feces even after a week of observation. Similarly, Aluoch *et al.* (2015) found no presence of *Pilobolus* sp. in rabbit feces, and the growth of *Pilobolus* sp. was only observed in zebra, waterbuck, impala, Thomson's gazelle,

hartebeest, buffalo, giraffe, muntjac, and eland feces. In addition, the presence of coprophyllous fungi can act as a monitoring tool for ecosystem changes. The level of diversity can indicate environmental stability; the higher the diversity, the more stable the environment, while low diversity indicates stress on the ecosystem.

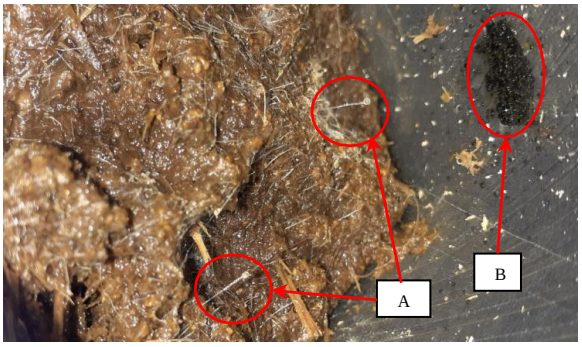

Figure 1. Growth of *Pilobolus* sp. on horse feces (A) *Pilobolus* sp.; (B) Spores of *Pilobolus* sp. (Source: Personal Documentation, 2021)

In Figure 1, it can be observed that the spores of *Pilobolus* sp. are attached to the walls of the container used. These spores accumulate in the area of the container where a gap for light was provided during incubation. According to Aluoch *et al.* (2015), the purpose of providing a light gap is to induce sporulation. Melnick (1996) states that the uniqueness of this fungus is its ability to shoot its spores, earning *Pilobolus* sp. the nickname "*shotgun fungi*". *Pilobolus* demonstrates a phototropic mechanism where its sporangia shoot the spores towards the direction of incoming light.

Morphology of *Pilobolus* sp. Fungi

In the six samples incubated for 4-7 days, namely horse, cow, pig, and buffalo feces, *Pilobolus* was predominantly found growing as fungi with a transparent fruiting body, stalk, and cap. Additionally, there were black spots on the uncovered part of the cap (Figure 1B; Figure 2), which represent the spores of *Pilobolus* sp. This is consistent with the reported morphology of *Pilobolus* sp. by Mulyani (2004), stating that the morphology of *Pilobolus* fungi consists of spores, sporangium, sporangiophore, and hyphae. *Pilobolus* fungi have black, egg-shaped sporangia that, when ruptured, release numerous round spores. According to Rajachan et al. (2014), the sporangiophore measures between 1.7 to 3.5 mM, is yellowish in the subsporangial swelling and has a smooth columellate sporangium wall, 70–124 µm in diameter; sporangiospores produced in the sporangium are oval to elliptical, 4.5–7.5 × 5.5–9.5 µm.

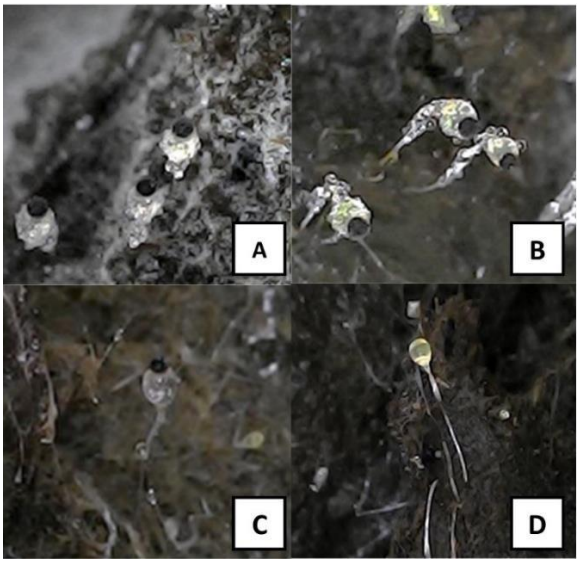

Figure 1 *Pilobolus* sp. from **a**. cow, **b**. horse; **c**. pig, **d**. buffalo

Pilobolus has a morphology characterized by a transparent stalk with a black sporangium on top. As it grows, the fruiting body of *Pilobolus* has a light-sensitive region, causing it to orient itself towards the incoming light (Greaves, 2014).

CONCLUSION

Based on the exploration of *Pilobolus* sp. fungi from various livestock feces, it can be concluded that *Pilobolus* sp. can be found in four different types of feces, namely horse, cow, buffalo, and pig, with varying growth times.

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