Detection of Psychotropic Compound in Coprophilous Fungi in District of Baturraden Banyumas Regency

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Abstract
Coprophilous fungi or dung-loving fungi are fungal group which adapted to life on dung and fecal of herbivorous animals. Coprophilous fungi contain psychotropic compounds, substances or drugs, both natural and synthetic, but not narcotics. This compound comprises psychoactive properties that cause selective influence on the central nervous system and lead distinctive changes in mental activity and behavior. Chemical Spot Test still remain an important method for the preliminary identification of illicit drugs and other psychotropic compound in spite of developments in instrumental technology which enables to use in field. Banyumas Regency, especially Baturraden district is a very potential habitat for coprophilous fungi due to a lot of cattle farms were established there. In addition, the environmental condition also suitable for the fungal growth. This study aim to make an inventory and identify the coprophilous fungi that found in District of Baturraden Banyumas Regency and detect the presence of psychotropic compound in that fungi. The research will be conducted using purposive random sampling and Color Test or Chemical Spot Test analysis. The obtained data is analyzed descriptively by comparing with Atlas of The Munsell Color System. This research obtained seven genera of coprophilous fungi i.e: Coprinellus sp., Coprinopsis sp., Entoloma sp., Gymnopus sp., Lepiota sp., Parasola sp. and Stropharia sp. that discovered in two cattle farms in Baturraden District.

Key Words: coprophilous, fungi, psychotropic

INTRODUCTION
Coprophilous fungi or dung-loving fungi are an artificial group of fungi which adapted to life on dung and fecal pellets of herbivorous animals. Coprophilous fungi release their spores to the surrounding vegetation, which are then eaten up by herbivores animals. The spores remain in inside the animal along the plants are digested, passed through the animal intestines, and finally defecated. The fruiting bodies of the fungi then grow from the animal feces. The spores themselves survive from digestion process because protected by thick-cell wall and later allowing them to germinate in the dung without competition from other organisms. Coprophilous fungi play an essential role in the ecosystem. They are responsible for recycling the nutrients in animal feces through decomposition of cellulose and lignin (Khiralla, 2007; Mohammed et al., 2017).

Coprophilous fungi also contain psychotropic compounds, substances or drugs, both natural and synthetic, but not narcotics. This compound has psychoactive properties which cause selective influence on the central nervous system and distinctive changes in mental activity and behavior. Generally coprophilous fungi contain psilocin and psilocybin that causing hallucination. The hallucination effect from coprophilous fungi varies depending on the gender, age, activity, dose, exposure method, feeling, body posture and metabolic ability (Sholihah, 2013; Suaniti et al., 2018).

Chemical Spot Test remains an essential tool for the preliminary identification of illicit drugs and psychotropic compounds despite developments of portable instrument technology are massive and enables to use in the field. Several reagents are used in the chemical spot test. Meanwhile, this study used Ehrlich's reagent and Marquis reagent. Ehrlich's reagent is used to detect tryptamine and Lysergic Acid Diethylamide, while the Marquis reagent is the primary test for opiates, hallucinogens, and amphetamine type compounds (Toole et al., 2007).

District of Baturraden because the study about the fungi are still rare in Indonesia. Therefore, the purposes of this research are to make an inventory and identification of coprophilous fungi in Baturraden District and detect the psychotropic compound from coprophilous fungi which obtained from Baturraden District. Furthermore, the research is expected to provide information about coprophilous fungi and their psychotropic compounds.
RESEARCH METHOD

Material, Location, and Time of Research
The materials which are used in this research include Coprophilous fungi taken from the sampling site in cattle farm in District of Baturraden Banyumas Regency, Alcohol 70%, p-Dimethylaminobenzaldehyde (C₉H₁₁NO), Methanol 96% (CH₃OH), Phosphoric acid (H₃PO₄), Glacial acetic acid (CH₃COOH), Formaldehyde (CH₂O) 40% and Sulfuric acid (H₂SO₄).

The tools which are used in this research include mushroom identification software Mycokey 4.1, National Audubon Society Field Guide to North American Mushroom identification book, thermohygrometer, soil tester, oven, vacuum pump, plastic bags, plastic jar, tissue paper, label, ceramic bowl, spoon, pipette, loop, aluminium foil, blender, mortar and pestle.

Location and Research Time Period
The sampling was taken place at cattle farms in District of Baturraden Banyumas Regency (Figure 1.). Research has been held at the Mycology and Phytopathology Laboratory of the Faculty of Biology Jenderal Soedirman University. The research was conducted for 5 months starting from November to March 2021.

Research Design

Survey Method
This research was conducted using purposive random sampling, which determines two villages in District of Baturraden whereas in each village specify one location; and Chemical Spot Test analysis to detect the psychotropic compound in the sample (Mumpuni et al., 2018; Knowlton et al., 1961). The sampling locations were cattle farm in Kutasari and Pamijen Village. Coprophilous identification were conducted on 06 November 2020, 12 February 2021, 04 and 18 March 2021 (Table 3.1).

Research Procedure

Sampling of Coprophilous Fungi
Macroscopic coprophilous fungi that appeared at the sampling site were collected and count individually then grouped according to their character. The collected fungi were put into sampling box and taken to the laboratory to identified.

Observation of Environmental Conditions
The temperature and pH of the substrate at each location was measured. Temperature and humidity was measured using thermohygrometer, while soil pH and soil humidity were measured using soil tester.

Identification of Coprophilous Fungi
The Coprophilous identification was conducted according to the macromorphology characteristics i.e. fruit body shape, cap color when young and mature, cap diameter, upper and lower shape of cap, cap surface, cap edge, cap margin, hymenophore type (lamellae, pores, teeth) including: how to attach to the stipe, length, distance between rows, and margins (Putra, 2020). Identified characters are compared to the Identification was done by comparing the fungi characteristics to National Audubon Society Field Guide to North American Mushrooms, An Identification Guide Psilocybin Mushrooms of the World and mushroom identification software Mycokey 4.1.

Table 1. Location, Coordinate and Date of Sampling

| Location            | Coordinate      | Date of Sampling |
|---------------------|-----------------|------------------|
| Kutasari Cattle Farm| -7.3804747, 109.2290143 | 06 November 2020, 12 February 2021, 04 & 18 March 2021 |
| Pamijen Cattle Farm | -7.3765028, 109.2297783  | 06 November 2020, 12 February 2021, 04 & 18 March 2021 |
Detection of Psychotropic Compounds
Detection of psychotropic compounds on coprophilous fungi was carried out by drying the fruit body and using the Chemical Spot Test/Color Test using the Ehrlich Reagent and the Marquis Reagent.

Drying Process of Coprophilous Fungi Fruit Body
Detection of psychotropic compounds on coprophilous fungi was carried out by drying the fruit body and using the Chemical Spot Test/Color Test using the Ehrlich Reagent and the Marquis Reagent

Detection of Psychotropic Compound Using Ehrlich Reagent (Garraway & Maharaj, 2007)
Ehrlich reagent was prepared by mixing 1 g of p-dimethyl-aminobenzaldehyde in 10 ml of 95% methanol and 10 ml of concentrated orthophosphoric acid. Small amount of test material was placed into porcelain bowl and added with two drops of Ehrlich reagent. After 15 minutes, a change in violet color to grey violet (based on the color table) indicated the availability of psilocybin or psilocin. The positive reaction for psilocybin content can be obscured by taking 2 drops of material sample and methanol mixing and placed on a spot test to evaporate. The obtained residue was dissolved by adding two drops of Ehrlich reagent.

Detection of Psychotropic Compound Using Marquis Reagents (Mahmood et al., 2010)
Marquis reagent was prepared by making two solutions of A1 and A2. Reagent A1 consist of 10 ml of glacial acetic acid and 8-10 drops 40% Formaldehyde added. Meanwhile, reagent A2 consist of 2 drops of sulfuric acid solution. A small amount of test material was placed on a spot plate and then added one drop of A1 reagent and two drops of A2 reagent.

Data Analysis
Data obtained from the detection of psychotropic compounds in coprophilous fungi were analyzed descriptively.

RESULT AND DISCUSSION
Identification Result
Sampling was conducted four times in different period times during 5 months and started from 04.30 AM to prevent fungal damage caused by exposure to sunlight and human/animal activity. The sampling sites were located in two different villages in Baturraden District, which in Kutasari Village and Pamijen Village.

The first sampling site, Kutasari Village, has wet tropical climate with an average temperature 23°C, humidity 93% and soil pH in 7. In Kutasari Village were found seven fungal species i.e. Coprinellus sp., Coprinopsis sp., Entoloma sp., Gymnopus sp., Lepiota sp., Parasola sp., and Stropharia sp. meanwhile, Pamijen Village has an average temperature 24°C, humidity 90% and soil pH in 6.2. In Pamijen Village, We found two fungal species i.e. Coprinopsis sp and Parasola sp.

The presence of the fungi was dominant in Kutasari cattle farm. According to the observation, this is caused by the proper structural construction of the cattle farm dung storage area that has roof and close to the trench where the water flows is suitable for fungal growth. Meanwhile in Pamijen cattle farm, the pile of dung is left exposed to the sun, doused and irrigated with a large amount of water, even though it is collected in a dung storage area.

Based on the sampling result in Table 2, Coprinopsis sp. which obtained from sampling area are very similar to Coprinopsis lagopus. It grew solitary or in group and appeared on feces and rice straw in dung shelter. The fruit body type is agaricoid, with 100 mm of length, 40 mm of average pileus width, and 8 mm of average stipe width. It has campanulate shape (bell-shaped) and very swooping upwards on the edge with greyish color, pileus surface is moist-radially fibrillose. Lamellae attachment is free with crowded spacing and greyish color. The stipe color is whitish to cream with absence of ring/annulus. In addition, the flesh is fragile. Referring to Amandeep et al. (2014), the fungi can be determined by the shape of

| Genera            | 06 November 2020 | 12 February 2021 | 04 March 2021 | 18 March 2021 |
|-------------------|------------------|------------------|---------------|--------------|
|                   | KS   | P    | KS    | P    | KS    | P    | KS    | P    |
| 1 Coprinellus sp. | ×    | ×    | ✓     | √    | ×     | ×    | ×     | ×    |
| 2 Coprinopsis sp. | ✓    | ✓    | ✓     | ✓    | ✓     | ✓    | ✓     | ✓    |
| 3 Entoloma sp.    | ×    | ×    | ×     | ✓    | ✓     | ×    | ✓     | ×    |
| 4 Gymnopus sp.    | ×    | ×    | ×     | ×    | ×     | ✓    | ×     | ×    |
| 5 Lepiota sp.     | ×    | ×    | ×     | ✓    | ×     | ✓    | ✓     | ✓    |
| 6 Parasola sp.    | ×    | ×    | ✓     | √    | ×     | ×    | ✓     | ✓    |
| 7 Stropharia sp.  | ✓    | ✓    | ✓     | ✓    | ×     | ×    | ✓     | √    |

*KS : Kutasari
**P : Pamijen
pilus that canpanulate (bell-like) when young and applanat (flat) when mature. The pilus surface is moist, firstly it is white in color and continuously changed to gray color that covering the entire pilus surface. Lamellae attachment is free to adnexed and also thin flesh. As stated by Grainger (1946) and Boddy et al. (2007), Coprinopsis sp. always found at all sampling locations due to their high tolerance to environmental conditions such as temperature, pH and humidity of substrate. Furthermore, the mycelia can grow aggressively in low nutrients environment. Therefore Coprinopsis sp. can grow and find dominate in one location.

Coprinellus sp. that found in the sampling site grow and appear on woods near the dung shelter. Fungi grow in the singular (solitary) and in groups. Fruit body type is agaricoid with 30-50 mm of average fruit body height, 30 mm of average pilus width and 1.5 mm of average stipe width. It has flat pilus with a whitish to cream color and brown spot on the center. The pilus surface dry-smooth without structure. Lamellae attachment is free with crowded spacing with buff to cinnamon color. The stipe color is whitish to cream with absence of ring/annulus. The flesh of this fungi is fleshy. Referring to Putra (2020), Gymnopous sp. found grow solitary or in small group. Pilus are flat, light brown, 17 mm in diameter, smooth surface without changes in color. The hymenophore is lamella, short decurrent in attachment to the stipe, medium rows, and crenate margin. Stipe are cylindrical-shaped, light brown in colour, 2 mm in diameter, 19-20 mm in length, and smooth surface. The stipe has hollow cross section without partial veil. This mushroom has soft and fleshy fruit body texture and does not have a distinctive odor.

Leptota sp. that found in Kutasari Village, grow in solitary and appear on soil near the cow cage. Fruit body type is agaricoid with 40 mm of height, 25 mm of pilus width and 3 mm of stipe width. It has flat pilus shape with whitish to cream color, pilus surface is dry-scaly. Lamellae attachment is free with crowded spacing, has whitish to cream color. The stipe color is whitish to cream with the presence of ring/annulus. The flesh of this fungi is fleshy. According to Putra et al. (2018), Leptota sp. found growing independently (solitary) on a substrate in the form of soil. The pilus is white with a black part in the middle. The pilus is 12-17 mm in diameter with a flat top when young and depressed when old and a round bottom (ovoid) in shape. Scaly pilus surface. The edges of the pilus are crisped with straight margins. This fungi has a hymenophoric type in the form of lamellae that is not attached to the stipe (free), the length of the lamella size is 0.5-0.8 cm. The stipe is equal, white, 0.1 cm in diameter, 2.75 cm long, smooth, attached to pilus in a central position, the type of attachment to the substrate is rhizoid with a ring/annulus and hollow. Cartilaginous fruit body texture without distinctive odor discovered in Kutasari Village cattle farm and Pamiyen Village cattle farm. grow in solitary with flat and conical pilus shape, and cream spot in the central of pilus. These characteristics similar to Parasola picatilis. According to Al-Khesraji (2018), the size of pilus up to 2.5 cm in diameter with oval then convex to flattened or umbrella-like of shaped at maturity. the pilus color is distinctive central brown disc grooved, grey or metallic grey, not deliquescent. The lamella is free, moderately separated, radiate from a collar around the stipe apex on the underside of the cap, black in age. Stipe is up to 7 cm long, white, hollow, equal, fibrillose. The spore print is black. This fungi is nonpoisonous and inedible.

Stropharia sp. from 06 November 2020, 12 February 2021 and 18 March 2021 sampling only found in Kutasari Village cattle farm. Stropharia sp.
is commonly known as *Psilocybe cubensis*. This species was first discovered in 1906 as *Stropharia cubensis* by Franklin Sumner Earle in Cuba. In 1907 it was identified as *Naematoloma caeruleum* in Tonkin by Narcisse Theophile Patouillard, while in 1941 it was named *Stropharia cyanescens* by William Alphonso Murrill in Florida. Then finally the name was equated to the species *P. cubensis* (Stamets, 1996). *Stropharia* sp. which discovered in Kutasari Village cattle farm had very similar characteristics to *P. cubensis*. Referring to Tsujikawa (2003), the pileus has conical to campanulate (bell shape) shape, whitish to yellow color and smooth surface. Also, the lamellae color is nearly black.

**Chemical Spot Test Result**

The fungi samples were tested using Chemical Spot Test with the use of Ehrlich reagent and Marquis reagent. Five fungi containing psychotropic compounds are *Coprinellus* sp., *Coprinopsis* sp., *Entoloma* sp., *Parasola* sp., and *Stropharia* sp. Two of them indicated to contain psilocybin or psilocin; *Coprinellus* sp. and *Coprinopsis* sp. Whereas the other three, *Entoloma* sp., *Parasola* sp., and *Stropharia* sp., contain 2C-B and amphetamine or methamphetamine.

Chemical Spot Tests are presumptive illicit drug identification techniques commonly used by law enforcement, border security personnel, and forensic laboratories. Chemical reagents are added to a small sample of the material and the color changes are observed with the naked eye. The color change specification will indicate the presence of a particular drug. The lack of sample preparation required, ease of use, rapid results afforded, portability, and low cost make chemical color tests an ideal presumptive identification. These attributes are beneficial considering the large numbers of samples received. Concerns regarding the selectivity of color testing are apparent and have received attention via numerous media reports (Philp & Fu, 2017).

The chemical spot tests taken in this research were presumptive tests using Ehrlich Reagent and Marquis Reagent. Ehrlich's reagent is a reagent containing p-dimethylaminobenzaldehyde (DMAB) which can act as an indicator to identify indole and urobilinogen. This reagent is used for medical purposes to determine, among others, providing a diagnosis of liver diseases, hemolytic processes, occlusion of the common bile duct, and carcinoid syndrome. In addition to its medical uses, Ehrlich's reagent can also be used to indicate psychoactive compounds such as tryptamine and Lysergic Acid Diethylamide (LSD). Also, this reagent shows positive results for opium test because of tryptophan in natural opium (Lamb et al., 2015; Yoho et al., 2017).

Marquis reagent is used to identify alkaloids and other compounds. Marquis reagent works with morphinan drugs, including formaldehyde, which reacts with the aromatic rings on tested chemicals and links them together. This reagent is used in the main presumptive test in testing for ecstasy and several other compounds such as cocaine, opiates (codeine, heroine) and phenethylamine (2C-B, mescaline). The test is done by scraping a small amount of the material and adding a drop of the reagent (which is initially clear and colorless). The results are analyzed by looking at the resulting mixed color, and at the time it takes for the color change to become noticeable (Darsigny et al., 2018).

Based on the table 4.3., result of Ehrlich reagent *Coprinellus* sp. and *Coprinopsis* sp. was changed color to red with tint of violet color this proved that these two samples contained psilocybin or psilocin. As stated by Mahmood et al. (2010), the Ehrlich reagent color will change from yellow color into violet or gray-violet indicated the presence of psilocybin or psilocin. The chemical spot test result of *Gymnopus* sp. and *Lepiota* sp. using Ehrlich reagent shows there were no change of color that appeared. Even though these fungi are not harmful, referring to Ulya et al. (2017), these fungi are not for consumed.

For other sample such as *Entoloma* sp. it also shows change of color from yellow into orange with tint of brown. For *Parasola* sp. the Ehrlich reagent turns into light brown and then for the *Stropharia* sp. it turns into dark orange. As stated by Philp & Fu (2017), since the Ehrlich reagent found excellent use a general screening test for many synthetic drugs, several color changes also shows different psychoactive compound such as orange-brown, black and olive-green color changes with amphetamine HCl, 3,4-methylenedioxyamphetamine HCl, and 3,4-dimethoxynmethamphetamine HCl. The chemical spot test result of *Coprinellus* sp. and *Coprinopsis* sp. using Ehrlich reagent turns into a dark reddish orange and dark red with slight tint of violet, which indicate the presence of psilocybin or psilocin (Mahmood et al., 2010). According to Arini et al. (2017) *Coprinellus* sp. especially *Coprinellus disseminatus* has potential as medicine. *C. disseminatus* has potential for curing breast cancer (Novakovic et al., 2016).

*Parasola* sp. from which are Based on Table 4.3, Marquis reagent from seven fungi samples only five genera that contain psychotropic compound. *Coprinellus* sp., *Coprinopsis* sp., and *Entoloma* sp. show the color turn into light yellow with tint of brown, deep yellow and yellow with tint of brown.
This indicated that these 3 fungi may contain methamphetamine. Meanwhile for Stropharia sp. shows color change into yellow with tint of green indicated that it contained 2,5-dimethoxy-4-bromophenethylamine (2C-B) (Toole et al., 2007). In addition, these fungi also has high nutritional content, such as protein, fats, fatty acids, vitamins, minerals, free sugars, tocopherols, organic acids and phenolic acids (Noverita et al., 2018; Stojkovic et al., 2013). As shown in the result, however the Ehrlich reagent result of Coprinellus sp. and Coprinopsis sp. contain psilocybin or psilocin, in Marquis reagent result shows that these two fungi contain methamphetamine. Methamphetamine (N-methyl-1-phenylpropan-2-amine) include to group I narcotics. This compound is synthesized and can be a white, yellow or brown crystalline powder and can also be in the form of pharmaceutical preparations (tablets, capsules, caplets). Its use is usually by inhalation (inhaled through the nose), intravenously and orally. Referring to Petit et al. (2012), the usage of methamphetamine will show several clinical effects such as accelerates heart rates, raises blood pressure and temperature, pupil dilatation, gives positives subjectives effects and mood, remove tiredness, brings power, euphoria and self-control.

2C-B or 2,5-dimethoxy-4-bromophenethylamine or Nexus is psychedelic drugs derived from the 2C family. This drug was first synthesized by Alexander Shulgin in 1974. 2C-B was intended to replace ecstasy/MDMA when it was banned. This drug is in the form of a white powder which is sometimes packaged as tablets or gel caps. This medicine is usually taken orally, inhaled (insuffalated) or vaporized. As proven by Papaseit et al. (2018), consuming 2C-B will affect increasing blood pressure and heart rate, hallucinating, mixed euphoric and psychedelic experience consisting of a temporary altered state of consciousness.

Qualitative presumptive analysis for drug samples indicates the presence or absence of a particular drug class using simple methods often amenable to portable field test kits. These simple methods need further analysis about the usage of the substance contained sample. Followed by a confirmatory instrumental analysis uses high discriminating power techniques performed in a laboratory to accurately identify all components, quantify drugs present, and profile chemical impurities (Philp & Fu, 2017). For example, some species of Gymnopus sp. has several usages such as including edible fungi, poisonous, antibacterial and antifungal (Dai, 2009 in Ramadhani et al., 2019).

Table 2. Result of Chemical Spot Test Containing Psychotropic Compound

| No. | Type of Fungi | Ehrlich Reagen | Marquis Reagen |
|-----|--------------|----------------|---------------|
|     |              | Atlas Munsell Color Chart | Test Result | Atlas Munsell Color Chart | Test Result |
| 1   | Coprinellus sp. | Dark Orangish Red 5R 5/14 | + | Light Yellowish Brown 10Y 7/4 | + |
| 2   | Coprinopsis sp | Dark Red 5R 3/10 | + | Deep Yellow 7.5YR 6/12 | + |
| No. | Species        | Color Change          |
|-----|----------------|-----------------------|
| 3   | *Entoloma* sp. | Orange to Brown 7.5YR 6/14 | Yellowish Brown 5YR 6/14 |
| 4   | *Gymnopus* sp. | No Color Change        | No Color Change          |
| 5   | *Lepiota* sp.  | No Color Change        | No Color Change          |
| 6   | *Parasola* sp. | Light Brown 5YR 5/8    |                          |
| 7   | *Stropharia* sp.| Dark Orange 7.5YR 3/8  |                          |
CONCLUSION

The identification result of coprophilous There are 7 species of Coprophilous fungi that have been found from Kutasari and Pamijen cattle farm village. The founded Coprophilous fungi are Coprinus sp., Coprinopsis sp., Entoloma sp., Gymnopilus sp., Lepiota sp., Parasola sp., and Stropharia sp.. Five fungal species contain psychothropic compounds where Coprinellus sp. and Coprinopsis sp. contain psilocybin or psilocin, while Entoloma sp., Parasola sp., and Stropharia sp. contain 2C-B and amphetamine or methamphetamine.

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