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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 psylocibin and psylocin 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.

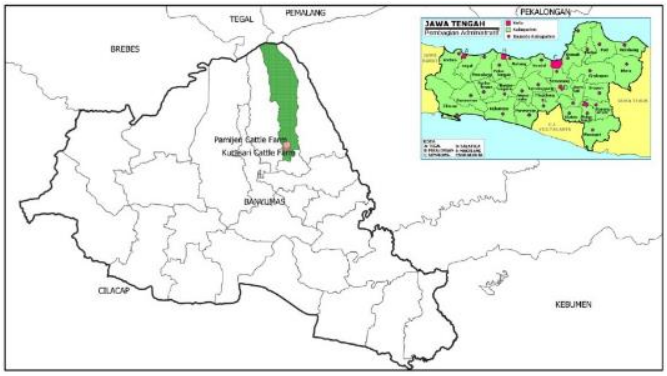

Figure 1. Sampling Site inBaturraden District

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-Dimethyl-aminobenzaldehyde ($C_9H_{11}NO$), Methanol 96% (CH_3OH), Phosphoric acid (H_3PO_4), Glacial acetic acid (CH_3COOH), Formaldehyde (CH_2O) 40% and Sulfuric acid (H_2SO_4).

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, pippete, 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 ortho-phosphoric 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^{o}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^{o}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

Table 1. Result of Sampling in Baturraden District

	Genera	06 November 2020		12 Febuary 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

pileus that campanulate (bell-like) when young and applanate (flat) when mature. The pileus surface is moist, firstly it is white in color and continuously changed to gray color that covering the entire pileus surface. Lamella 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 pileus width and 1.5 mm of average stipe width. It has flat pileus with a whitish to cream color and brown spot on the center. The pileus 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 soft and thick/fleshy. According to Putra (2020), *Coprinellus* sp. is grow on humus soil. Pileus are white to cream in color with 3-6 mm of diameter and radially thread like surface. The hymenophore is lamella 5 mm in length, crowded rows, and entire margins. The stipe has white color with rooting in form and smooth surface with 12 mm in length. The stipe has hollow stipe cross section without partial veil. This mushroom has a soft and fleshy fruit body texture and has distinctive odor like the smell of vegetables with butter taste.

Entoloma sp. that found is grow and appear on soil near the cow cage. Growth in singular. Fruit body type is agaricoid, body fruit size 40 mm, average pileus width 30 mm and average stipe width 9 mm. It has flat pileus with whitish color, pileus surface slimy-smooth without structure. Lamellae attachment is free with crowded spacing, has clay pink to rose color, The stipe color is whitish to cream with absence of ring/annulus. The flesh is soft and fleshy. Referring to Kondo *et al*. (2017), pileus 50 to 100 mm in diameter with pileus shape convex or flat to depressed. Pileus colors are varied from grayish brown, yellowish gray to brown or reddish brown when mature and were hygrophanous. Gills were primarily white and pinkish or brown when old. Stipes were 4 to 12 cm long and 5 to 15 mm wide. The flesh mostly fragile but sometimes solid.

Gymnopus sp., grow solitary and appear on soil near the cow cage. The fruit body type is agaricoid with 30 mm of height, 20 mm of pileus width and 10 mm of stipe width. It has flat pileus shape with dull brown color, pileus surface is dry-smooth no structure. Lamellae attachment is free with crowded spacing and dull brown color. The stipe color is buff to cinnamon with absence of ring/annulus. The flesh of this fungi is fleshy. Referring to Putra (2020), *Gymnopus* sp. found grow solitary or in small group. Pileus 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.

Lepiota 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 pileus width and 3 mm of stipe width. It has flat pileus shape with whitish to cream color, pileus 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), *Lepiota* sp. found growing independently (solitary) on a substrate in the form of soil. The pileus is white with a black part in the middle. The pileus is 12-17 mm in diameter with a flat top when young and depressed when old and a round bottom (ovoid) in shape. Scaly pileus surface. The edges of the pileus 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 pileus 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 Pamijen Vilage cattle farm. grow in solitary with flat and conical pileus shape, and cream spot in the central of pileus. These characteristics similar to *Parasola plicatilis*. According to Al-Khesraji (2018), the size of pileus up to 2.5 cm in diameter with oval then convex to flattened or umbrella-like of shaped at maturity. the pileus 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 caerulenscens* in Tonkin by Narcisse Theophille 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 Dyethilamide (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-methylenedioxyamphe-tamine HCl, and 3,4-dimethoxymethamphetamine 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-bromophenethyl-amine 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 (insuffalted) 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	+

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 *Coprinellus* sp., *Coprinopsis* sp., *Entoloma* sp., *Gymnopus* sp., *Lepiota* sp., *Parasola* sp., and *Stropharia* sp.. Five fungal species contain psycothropic 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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