

Antimicrobial Assay of Soil Mold Isolates from Wonorejo Surabaya

Septia Arisanti¹, Nengah Dwianita Kuswytasari¹, and Maya Shovitri¹

Abstract—This study was aimed to examine antimicrobial activity of 34 soil molds isolates from the Wonorejo Surabaya on the growth of Gram negative bacteria (*Escherichia coli* and Coliform Bacteria Group), Gram positive bacteria (*Bacillus subtilis*) and yeast (*Saccharomyces cerevisiae*). Antimicrobial ability detected with modification of dual culture antagonism assay in Potato Dextrose Agar (PDA) medium. The result showed that genus *Aspergillus*, *Scopulariopsis*, *Penicillium*, *Paecilomyces*, *Fusarium*, and *Trichoderma* were able to inhibit *E. coli*; while genus *Aspergillus*, *Scopulariopsis*, *Penicillium*, *Paecilomyces*, *Exophiala*, *Stachybotrys*, and *Acremonium* inhibit *B. subtilis*; further on only genus *Aspergillus* could inhibit group of Coliform bacteria; and genus *Scopulariopsis*, *Penicillium*, *Trichoderma*, and *Absidia* inhibited the growth of yeast *S. cerevisiae*.

Keywords—Antimicrobial assay, clearing zone, soil mold

Abstrak—Penelitian ini bertujuan untuk mengetahui kemampuan antimikroba 34 isolat kapang tanah Wonorejo Surabaya terhadap bakteri Gram negatif (*Escherichia coli* dan kelompok bakteri Coliform), bakteri Gram positif (*Bacillus subtilis*), dan yeast (*Saccharomyces cerevisiae*). Kemampuan antimikroba dideteksi dengan modifikasi uji antagonisme dual culture pada medium Potato Dextrose Agar (PDA). Hasil menunjukkan bahwa genus *Aspergillus*, *Scopulariopsis*, *Penicillium*, *Paecilomyces*, *Fusarium*, dan *Trichoderma* menghambat *E. coli*; genus *Aspergillus*, *Scopulariopsis*, *Penicillium*, *Paecilomyces*, *Exophiala*, *Stachybotrys*, dan *Acremonium* menghambat *B. subtilis*; hanya genus *Aspergillus* yang menghambat kelompok bakteri Coliform; dan genus *Scopulariopsis*, *Penicillium*, *Trichoderma*, dan *Absidia* menghambat yeast *S. cerevisiae*.

Kata kunci—uji antimikroba, zona bening, kapang tanah

I. INTRODUCTION

Microbes are microscopic organisms which is comprised of bacteria, fungi and viruses. In interaction with humans, these microbes exist on the offensive. Examples of pathogenic bacteria *Escherichia coli* and the *Coliform* bacteria can cause gastrointestinal illness [1]. Molds and yeasts cause disease because it produces toxins (mycotoxins) which infect the surface of the body and attack the tissues in the body [2].

One of the efforts to fight microbes is to use other microbes that have antagonistic properties (antimicrobial) as a nuisance or other microbial metabolic inhibitors. Antagonistic microbes have the ability to produce antimicrobial compounds. Antimicrobial compounds produced by microbes is generally a secondary metabolite that is not used for the growth process [3], but for self-defense and competition with other microbes to obtain nutrients, habitat, oxygen, light and others [4]. Antimicrobial compounds can be classified as an antibacterial or antifungal [5].

The microbes have the ability to produce antimicrobial and antimicrobial compounds are

bacteria, aktinomycetes, and molds [6-7]. Aktinomycetes and groups of bacteria, such as the group of lactic acid bacteria and Gram-positive bacteria have been widely studied and is known as a source of various antimicrobial compounds [8]. Soil fungi that have antimicrobial activity is the genus *Aspergillus*, *Penicillium*, *Paecilomyces*, *Trichoderma* [2], and *Fusarium* [9] Genus-genus other soil fungi are able to produce antimicrobial compounds is not widely studied. Thus, in accordance with the opinion [2], screening isolates mold new ground, especially from various regions in Indonesia still have to be made to determine its potential as an antimicrobial agent.

In research of [10], has been isolated and purified isolates of soil fungi from Wonorejo Surabaya. Isolates has become a collection of the Laboratory of Microbiology and Biotechnology Department of Biology, ITS. Wonorejo Surabaya is one of the mangrove areas in Indonesia and included in Surabaya East Coast area with muddy substrate types [11], which is rich in organic material needed to carry out metabolism soil fungi [12]. In comparison, [2] reported that the mangrove forest areas in Jakarta were fungus genus *Aspergillus*, *Paecilomyces*, *Penicillium*, and *Trichoderma* which have antimicrobial properties against bacteria and yeast. However the potency of soil fungi isolates Wonorejo Surabaya especially as antimicrobial was unknown, so

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this research developed to test its antimicrobial ability.

II. METHOD

A. Subcultures Isolates

1. Soil fungi isolates Wonorejo Surabaya and Yeast *Saccharomyces cerevisiae*.

Thirty four pure isolates of soil fungi Wonorejo Surabaya region (collection of the Laboratory of Microbiology and Biotechnology Biological ITS) consisting of 17 genus of fungi, namely *Aspergillus*, *Fusarium*, *Penicillium*, *Paecilomyces*, *Verticillium*, *Trichoderma*, *Scopulariopsis*, *Curvularia*, *Stachybotrys*, *Gliocladium*, *Gliomastix*, *acremonium*, *Chaetomium*, *Mortierella*, *Absidia*, *Exophiala* and *Cephalophora* inoculated into the first test tube containing solid medium Potato Dextrose Agar (PDA) slant and a Petri dish containing solid medium Potato Dextrose Agar (PDA) aseptically. Then incubated at room temperature (30°C) for 3 days. Furthermore, the soil fungus culture tubes kept in a refrigerator at 4°C for stock cultures. Soil fungi cultured in Petri dishes as work culture, incubated returned up to 7 days.

Pure isolates of *S. cerevisiae* inoculated into 2 test tubes containing solid medium Potato Dextrose Agar (PDA) slant aseptically in duplicate and incubated at room temperature for 24 hours. Furthermore, the culture tubes used for work, while the other tube for culture preparation are also stored in the refrigerator at 4 ° C.

2. Isolates of *Escherichia coli* and *Bacillus subtilis* Bacteria

Bacterial isolates of *E. coli* and *B. subtilis* (collection of the Laboratory of Microbiology and Biotechnology Department of Biology ITS) was inoculated into 2 test tubes containing solid medium Nutrient Agar (NA) slant in duplo and aseptically, then incubated in an incubator (Mettler, BE-300 ®, Germany) at 37°C for 24 hours. Furthermore, the tube used for work, while the other tube stock cultures stored in a refrigerator at 4°C.

B. Culture Test Preparation

1. Inoculum Bacteria *Escherichia coli* and *Bacillus subtilis*

One loop aseptically cultured isolates from working 24 hours old were inoculated in 50 ml of Nutrient Broth (NB) medium in a 100 ml Erlenmeyer. Then incubated at room temperature (\pm 30°C) with shaking at 100 rpm on a rotary shaker (Health, HM-SR ®) for 24 hours, then performed for each bacterial dilution storied opponent in sterile distilled water. Then both starter inoculum of bacteria *E. coli* and *B. subtilis* cell number was calculated using the Haemocytometer Improved Neubauer to obtain the number of cells by 10^6 cells/ml.

2. Inoculum of *Coliform* Bacteria Group

Aseptically 1 ml samples of river water taken from the Kali Mas in Achievement Park Surabaya, inoculated in 9 ml medium in duplicate as BGLBB stock culture and work culture. Then incubated in an

incubator (Mettler, BE-300 ®, Germany) at 37°C for 24 hours. Then for *Coliform* bacteria group stock cultures stored in the refrigerator at 4°C, and for the culture of *Coliform* bacteria group work culture cells number calculated using a Haemocytometer Improved Neubauer to obtain the number of cells by 10^6 cells/ml.

3. Inoculum Yeast (*Saccharomyces cerevisiae*)

Suspension *S. cerevisiae* was prepared by inoculating one loop of pure isolates into 100 ml YEMES broth and incubated at room temperature (\pm 30°C) with shaking at 100 rpm on a rotary shaker (Health, HM-SR ®) for 24 hours [13]. The number of cells *S. cerevisiae* is calculated by enumerating the Haemocytometer Improved Neubauer to obtain the number of cells by 10^6 cells/ml.

C. Growth Inhibition Test

The test is performed by the modified method of antagonism dual culture. The assay to detect secretion of antimicrobial compounds from fungi isolates and their effects on the growth of bacteria and yeast opponent.

Gram negative bacteria used *E. coli* and *Coliform* Bacteria Group, whereas Gram positive bacteria used *B. subtilis*, for representatives of fungi used *S. cerevisiae*. Sterile cotton swab dipped in a suspension of *E. coli*, *B. subtilis*, the *Coliform* bacteria, and *S. cerevisiae* with each cell concentration of 10^6 cells/ml. Then by using the swab (swab method) was inoculated on PDA solid medium in Petri dishes. Similarly one colony isolates were inoculated soil fungi Surabaya Wonorejo 1 cm diameter in the center of a Petri dish. Petri dishes were incubated at room temperature for 7 days. Control treatments in the form of a Petri dish inoculated with bacterial isolates only test and a Petri dish inoculated soil fungi Wonorejo Surabaya alone. Observations about the presence or absence of clear zone, clear zone diameter and the diameter of each mold colony were done every 24 hours for 7 days. The ratio between the diameter and the diameter of the mold colonies's clear zone called growth inhibition opponent microbial isolates ratio as an indicator secretion of antimicrobial compounds of soil fungi isolates from Wonorejo Surabaya.

D. Research Design and Data Analysis

Each soil fungi isolates from Wonorejo Surabaya tested antimicrobial against four opponent microbes, each performed three replicates and data were analyzed descriptively. The parameters were observed in the presence or absence of clear zone around soil fungi colony, diameter of mold colony, diameter of clear zone, and ratio of clear zone were counted every 24 hours for 7 days incubation period.

III. RESULTS AND DISCUSSION

Based on the parameters of clear zone is seen that there were 23 isolates of soil fungi from Wonorejo that are inhibiting growth of fourth microbial opponent. Figure 1 dan Table 1 shows the value of clear zone ratio. The greater ratio of clear zone,

assumed greater the ability of the mold soil to inhibit the growth of opponent microbes.

According [14-15], clear zone is an indication of the absence or inhibition of microbial growth due to the excretion of antimicrobial compounds by other microbes opponent that are antagonistic. Clear zone around the colonies of soli mold seen since the first day or 24 hours of incubation, and then the next day covered by the growth of soil mold colonies. Soil fungi colony growth during the incubation period varies, depending on the speed of growth of each isolate soil fungi.

From 34 isolates of soil fungi were tested antimicrobial Wonorejo, there were 11 isolates were not able to inhibit the growth of microbes fourth opponent. This is not because the 11 isolates of fungi is not potentially inhibit all species of bacteria and yeast, but can be caused by microbial species opponent. According [5], each microbial species showed different susceptibility to antimicrobial compounds. So that in other bacteria and yeast species than those used in this test, the response could be different, which may be stunted due to resistance to antimicrobial compounds produced by soil fungi isolates.

Generally seen that the *B. subtilis* bacteria isolates greatest inhibited growth by a soil fungus *Exophiala* sp. (T3.8), *Acremonium* sp. (T3.9), *A. fumigatus* (T1.a6), and *A. versicolor* (T1.p3). Value ratio of the largest clear zone on each isolate soil fungi occurred on the first day of incubation. This is because the diameter of the mold colony on the first day of incubation still measuring ± 1 cm. Furthermore, the diameter of the mold colony growing following the long period of incubation. While on the other hand the diameter of clear zone formed from the first day until the last day incubation period is relatively constant, thus minimizing the value of the ratio of the clear zone.

Soil mold isolates from Wonorejo inhibited the growth of both *E. coli* and *B. subtilis* bacteria. There were 11 mold isolates be antimicrobial against *E. coli* and 20 mold isolates againts *B. subtilis*. From Figure 1 also shows that there are only 2 mold isolates were able to inhibit the growth all of 3 opponent bacteria (*E. coli*, *B. subtilis*, and the *Coliform* bacteria group), which isolates *A. niger* (T2.1) and *A. versicolor* (T1.p3).

Generally, the genus *Aspergillus* can inhibit the growth of bacteria *E. coli* and *B. subtilis*, but not able to inhibit the yeast *S. cerevisiae*. However, for mold isolates *A. niger* (T2.1) and *A. versicolor* (T1.p3) could inhibit the *Coliform* bacteria. Visible clear zone around the colony mold isolates *A. versicolor* (T1.p3) while inhibiting the growth of bacteria *E. coli*, *B. subtilis* and the *Coliform* bacteria is shown in Figure 1a, b, c, while at *A. versicolor* (T1.p3) were tested against the yeast *S. cerevisiae* does not seem clear zone formed (Figure 2d).

There are several isolates of mold that has the greatest ability to inhibit the growth of bacteria opponents characterized by the ratio of the value of

the largest clear zone between the isolates of other species. The ratio of clear zone mold isolates *A. versicolor* (T1.p3) against the *E. coli* bacteria was 2.5 cm, mold isolates *A. fumigatus* against *B. subtilis* at 3.24 cm, and mold isolates *A. niger* (T2.1) and *A. versicolor* (T1.p3) against *Coliform* bacteria in similar of 1.4 cm.

The results are consistent with the [16-17], who reported that the mold *Aspergillus* isolated from soil capable of producing antimicrobial compounds that can inhibit the growth of Gram positive and negative bacteria. And [18-19] also state that *A. flavus* produces aflatoxin antimicrobial compounds, and *A. niger* produces malformin and *A. fumigatus* produces helvolic acid or fumigacin.

Also according [17], generally antimicrobial compounds produced by *Aspergillus* are neutral, polar, and has a phenol group. Phenol is capable denaturated protein on the bacterial cell wall and membrane [20]. Research on soil mold on the growth inhibition of *Coliform* bacteria group has not done yet and couldn't gained much information about it. According [21] *Coliform* bacteria as a group of rod-shaped, Gram-negative, non-spore-forming, aerobic and facultative anaerobic ferment lactose and produce acid and gas within 48 hours at 35°C. At least soil fungi isolates from Wonorejo were able to inhibit the *Coliform* bacteria made possible by the presence of different types of bacteria (not consisting of one species of bacteria) that live together in groups, so the secondary metabolites produced by soil mold isolates from Wonorejo difficult to inhibit the growth of those who lived with in one group.

Isolates of *Scopulariopsis* sp. 1 (T2.19) inhibits bacteria *B. subtilis* and the yeast *S. cerevisiae*, while *Scopulariopsis* sp. 2 (T3.2) inhibits bacteria *E. coli* and *B. subtilis*. None of the genus *Scopulariopsis* isolates were able to inhibit the *Coliform* bacteria. The difference in the ability to inhibit microbial opponents, might caused by differences of these two species of *Scopulariopsis* isolates. When compared with *Aspergillus*, *Scopulariopsis* seen slower growth. This was conclude from the no clear zone seen by the growth of mold isolates colony until the seventh day of incubation.

Scopulariopsis sp. able to inhibit the growth of Gram positive and negative bacteria [22]. This is caused by *Scopulariopsis* sp. able to produce antimicrobial compounds trichothecenes [23], which has a mechanism of inhibiting protein synthesis and bacterial cell DNA [24].

Three soil mold isolates *Penicillium* sp. 2 (T1.a2), *Penicillium* sp. 3 (T1.q1) and *Penicillium* sp. 4 (T3.f2) could inhibit the growth of bacteria *E. coli* and *B. subtilis*. All isolates of the genus *Penicillium* are not able to inhibit the *Coliform* bacteria, and *Penicillium* sp.4 (T3.f2) were able to inhibit the yeast *S. cerevisiae*. While *Penicillium* sp. 1 (T4.e3) is not able to inhibit all microbial opponents. This suggests that species differences affect the ability to inhibit a microbial opponent.

Penicillium capable of producing the antibiotic penicillin to inhibit the synthesis of bacterial cell wall peptidoglycan [19,25]. *Penicillin* inhibits bacterial cell wall synthesis by inhibiting the synthesis of the enzyme or inactivation of the enzyme to synthesize peptidoglycan which an essential component of the bacterial cell wall. Inhibition of peptidoglycan synthesis causes loss of viability and often cause bacterial cell lysis. In addition, [26] states that the *Penicillium* sp. griseofulvin produce antimicrobial compounds that are inhibiting the growth of fungi [27], by interfering with the function of thread spindles and cytoplasmic microtubules, thus inhibiting fungal cell mitosis [28].

Five isolates of *Paecilomyces* seen that there are four isolates *Paecilomyces* sp. 1 (T2.11), *Paecilomyces* sp. 2 (T2.8), *Paecilomyces* sp. 3 (T2.20) and *Paecilomyces* sp. 5 (T4.6), which inhibits the growth of bacteria *B. subtilis*. Just *Paecilomyces* sp. 5 (T4.6) in addition to inhibiting the growth of *B. subtilis*, is also able to inhibit the growth of bacteria *E. coli*. All isolates of *Paecilomyces* sp. not able to inhibit the growth of *Coliform* bacteria and yeast *S. cerevisiae*. While the *Paecilomyces* isolates *Paecilomyces* sp. 4 (T3.1) are not able to inhibit all microbial opponents.

Paecilomyces reported to have antibacterial ability against *E. coli* [29], as well as generating Paecilospirone which inhibiting the growth of *B. subtilis* bacteria [19]. *Paecilomyces* can also produce antibiotic cephalosporin C which capable of inhibiting the growth of Gram-negative and positive [30]. Cephalosporin C has a mechanism of action similar to penicillin in inhibiting the growth of bacteria.

Based on the observations shows that soil mold isolates from Wonorejo the *Fusarium* sp. (T1.p2) is only able to inhibit the growth of *E. coli*. The ratio of the largest clear zone was seen only on the first day of incubation. Furthermore, due to rapid growth of *Fusarium* colonies, the clear zone can not be observed.

Fusarium that has been isolated from soil have antibacterial capabilities [22]. *Fusarium* produce antimicrobial compounds called trichothecenes, which has a mechanism of inhibiting protein synthesis and bacterial cell DNA [31].

Isolates Wonorejo soil fungi *Trichoderma* sp. 2 (T3.b1) could inhibit the growth of *E. coli* bacteria and the *Saccharomyces cerevisiae* yeast, but not for the *B. subtilis* bacteria and the *Coliform* bacteria. Isolates of *Trichoderma* sp. 1 (T2.13) does not have the ability to inhibit the growth of all microbes opponent.

Trichoderma sp. can produce Isocyanide-3-(-isocyanocyclopent-2-enylidene) propionic acid and Gliotoksin [32] to inhibit the growth of *E. coli* bacteria. According to [33], *Trichoderma* sp. the enzyme 1,3-glucanase and chitinase that is antibiotics. Both enzymes are capable of destroying glucans and chitin which is a component of fungal hyphae wall.

Based on these observations, soil mold isolates from Wonorejo *Exophiala* sp. (T3.8) is only able to inhibit

the growth of *B. subtilis* (see Figure 3). The ratio of the resulting clear zone of 5.52 cm are on the first day of incubation, and clear zone remain visible until the seventh incubation period because the growth rate of this genus are relatively slow when compared to the genus *Aspergillus* and *Fusarium*.

Exophiala sp. able to inhibit Gram-positive bacteria to produce antimicrobial compounds Chlorohydroaspyrones A and B [34], and Exophilin [35]. However, the inhibitory mechanism of action of these compounds against Gram positive bacteria is still unknown.

Soil mold Isolates from Wonorejo *Stachybotrys* sp. 1 (T2.7) and *Stachybotrys* sp. 2 (T2.10) only able to inhibit the growth of *B. subtilis* and other opponents of the microbes. Clear zone is only visible when the *Stachybotrys* sp. 1 (T2.7) were tested against *B. subtilis*. The ratio of the largest zones are on the first day of incubation. *Stachybotrys* genus colony growth has been rapid. This is evident from the clear zone on isolates of *Stachybotrys* sp. 1 (T2.7) is seen on the first day of incubation only and isolates of *Stachybotrys* sp. 2 (T2.10) seen from the first to the second day of incubation.

According to [36] the genus *Stachybotrys* potential as biological control agents against Gram-positive bacteria. *Stachybotrys* sp. can produce antimicrobial compounds such as trichothecenes, which has a mechanism of inhibiting protein synthesis and bacterial cell DNA [24].

Gliomastix sp. 1 (T3.6) and *Gliomastix* sp. (T3.7) is able to inhibit the growth of *B. subtilis* bacteria. However, all isolates *Gliomastix* not able to inhibit the growth of microbes other opponents. *Stachybotrys* genus colony growth has been rapid. Clear zone on *Gliomastix* sp. 2 (T3.6) isolates is seen on the first day of incubation only and isolates *Gliomastix* sp. 1 (T3.7) seen from the first to the second day of incubation. So the ratio of the largest zones are on the first day of incubation. And [37] reported that *Gliomastix* able to inhibit pathogenic bacteria by producing volatile oils. But the volatile oils and how inhibitory mechanism against Gram-positive bacteria is still unknown due to little research on that.

Acremonium sp. (T3.9) is only able to inhibit the growth of *B. subtilis* bacteria and was not able to inhibit the growth of three other microbial opponent. *Acremonium* colony growth is relatively slow, because the clear zone measured from the first day until the seventh day of incubation.

Acremonium producing cephalosporin C which has a mechanism similar to penicillin in inhibiting the growth of Gram-positive bacteria [38]. Based on the observation shows that isolates *Absidia* sp. (T3.k2) is only able to inhibit the growth of yeast *S. cerevisiae*. The largest clear zone on the first day of incubation. Furthermore, since the growth of *Absidia* colony very quickly, so clear zone can not be observed at the end of the incubation period. *Absidia* sp. isolated from the soil able to produce antimicrobial compounds that can inhibit the growth of yeast. However, if the compound

has not gotten the information generated and how the mechanism in inhibiting the growth of yeast [26].

IV. CONCLUSION

1. The soil mold isolates from Wonorejo Surabaya has antimicrobial capabilities. From 34 isolates tested, there were 11 isolates to be antimicrobial against *E. coli* (Gram-negative), 20 isolates of the *B. subtilis* (Gram positive bacteria), 2 isolates of the *Coliform* group of bacteria (Gram negative) and 4 isolates against *S. cerevisiae* (yeast).
2. The soil mold isolates that were inhibit *E. coli* is genus *Aspergillus*, *Scopulariopsis*, *Penicillium*, *Paecilomyces*, *Fusarium*, and *Trichoderma* with the largest value of the ratio of clear zone from the genus *Aspergillus*, which isolates *A. versicolor* (T1.p3) of 2.5 cm.

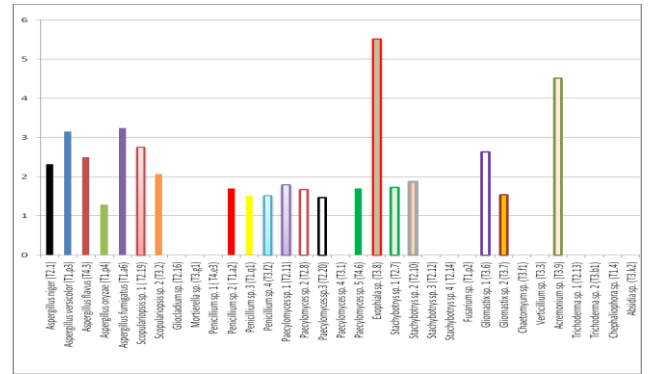
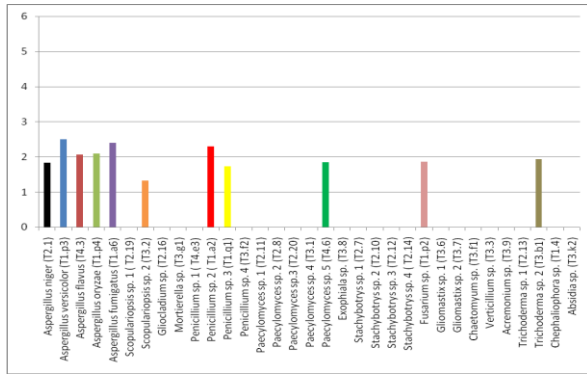
3. The soil mold isolates that were inhibit *B. subtilis* is a genus *Aspergillus*, *Scopulariopsis*, *Penicillium*, *Paecilomyces*, *Exophiala*, *Stachybotrys*, and *Acremonium* with the largest value of the ratio of clear zone from the genus *Exophiala* sp. (T3.8) isolates of 5.52 cm.

4. The soil mold isolates that were inhibit the *Coliform* Bacteria Group is derived from the genus *Aspergillus* isolates which were *A. niger* (T2.1) and *A. versicolor* (T1.p3) with the same ratio of clear zone is 1.4 cm.

5. The soil mold isolates that were inhibit yeast *S. cerevisiae* is the genus was *Scopulariopsis*, *Penicillium*, *Trichoderma* and *Absidia* with the largest value of the ratio of clear zone from the genus *Penicillium* isolates of *Penicillium* sp. 4 (T3.f2) of 1.88 cm.

TABLE 1.
THE RATIO OF CLEARING ZONE ZONE TO COLONY DIAMETER OF SOIL MOLD ISOLATES FROM WONOREJO SURABAYA

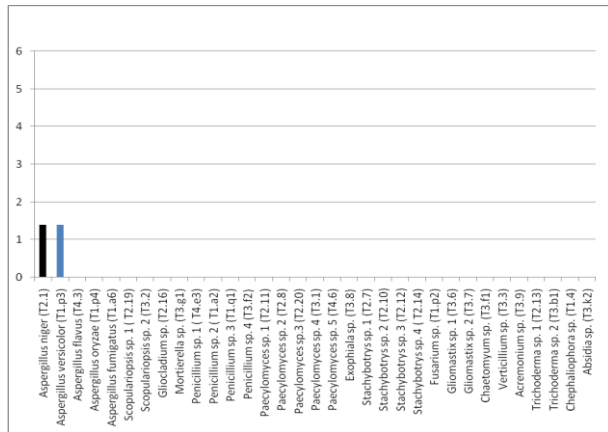
No.	Mold isolates	Code of isolates	Clearing zone ratio (cm)			
			<i>E. coli</i>	<i>B. subtilis</i>	<i>Coliform Bacteria Group</i>	<i>S. cerevisiae</i>
1.	<i>Aspergillus niger</i>	T2.1	1.84	2.32	1.4	-
2.	<i>Aspergillus versicolor</i>	T1.p3	2.5	3.15	1.4	-
3.	<i>Aspergillus flavus</i>	T4.3	2.06	2.5	-	-
4.	<i>Aspergillus oryzae</i>	T1.p4	2.1	1.29	-	-
5.	<i>Aspergillus fumigatus</i>	T1.a6	2.4	3.24	-	-
6.	<i>Scopulariopsis</i> sp. 1	T2.19	-	2.76	-	1.6
7.	<i>Scopulariopsis</i> sp. 2	T3.2	1.33	2.07	-	-
8.	<i>Gliocladium</i> sp.	T2.16	-	-	-	-
9.	<i>Mortierella</i> sp.	T3.g1	-	-	-	-
10.	<i>Penicillium</i> sp. 1	T4.e3	-	-	-	-
11.	<i>Penicillium</i> sp. 2	T1.a2	2.3	1.7	-	-
12.	<i>Penicillium</i> sp. 3	T1.q1	1.73	1.52	-	-
13.	<i>Penicillium</i> sp. 4	T3.f2	-	1.52	-	1.88
14.	<i>Paecilomyces</i> sp. 1	T2.11	-	1.8	-	-
15.	<i>Paecilomyces</i> sp. 2	T2.8	-	1.67	-	-
16.	<i>Paecilomyces</i> sp. 3	T2.20	-	1.47	-	-
17.	<i>Paecilomyces</i> sp. 4	T3.1	-	-	-	-
18.	<i>Paecilomyces</i> sp. 5	T4.6	1.85	1.7	-	-
19.	<i>Exophiala</i> sp.	T3.8	-	5.52	-	-
20.	<i>Stachybotrys</i> sp. 1	T2.7	-	1.73	-	-
21.	<i>Stachybotrys</i> sp. 2	T2.10	-	1.87	-	-
22.	<i>Stachybotrys</i> sp. 3	T2.12	-	-	-	-
23.	<i>Stachybotrys</i> sp. 4	T2.14	-	-	-	-
24.	<i>Fusarium</i> sp.	T1.p2	1.86	-	-	-
25.	<i>Gliomastix</i> sp.1	T3.6	-	2.63	-	-
26.	<i>Gliomastix</i> sp.2	T3.7	-	1.54	-	-
27.	<i>Chaetomyum</i> sp.	T3.f1	-	-	-	-
28.	<i>Verticillium</i> sp.	T3.3	-	-	-	-
29.	<i>Acremonium</i> sp.	T3.9	-	4.52	-	-
30.	<i>Trichoderma</i> sp.1	T2.13	-	-	-	-
31.	<i>Trichoderma</i> sp.2	T3.b1	1.94	-	-	1.63
32.	<i>Cephaliphora</i> sp.	T4.2	-	-	-	-
33.	<i>Curvularia</i> sp.	T1.4	-	-	-	-
34.	<i>Absidia</i> sp.	T3.k2	-	-	-	1.22



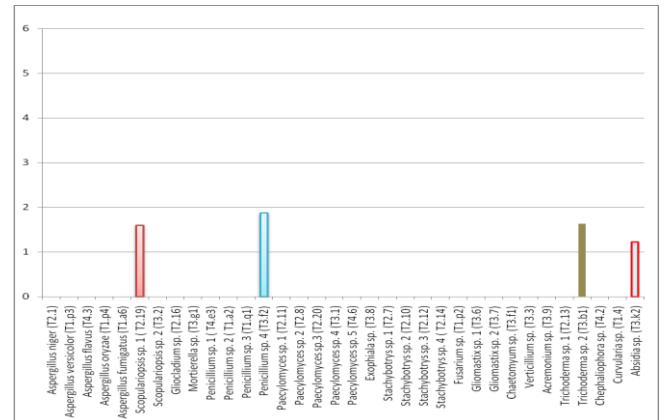
Description: - there is no clear zone, which means the soil mold isolates from Wonorejo Surabaya is not able to inhibit the growth of microbes opponant

(a)

(b)



(c)



(d)

Figure 1. Graph ratio clear zone (cm) (on the x axis) of soil mold isolates from Wonorejo Surabaya (on the y-axis) in inhibiting the growth of (a) *E. coli* bacteria, (b) *B. subtilis* bacteria, (c) *Coliform* bacteria group and (d) *S. cerevisiae* yeast.

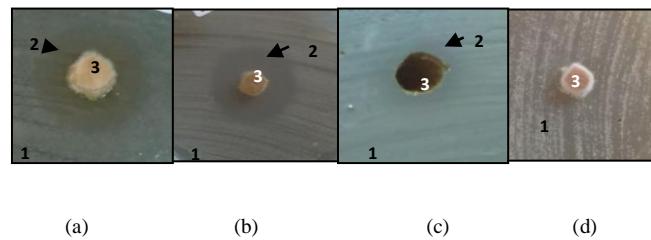


Figure 2. *A. versicolor* (T1.p3) against (a.) *E. coli*, (b.) *B. subtilis*, (c.) *Coliform* bacteria group, (d.) *S. cerevisiae* yeast on the first day of incubation with the description: 1. Microbial opponant, 2. clear zone, 3. isolates fungus *A. versicolor*.

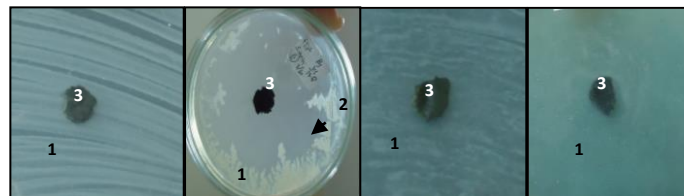


Figure 3. *Exophiala* sp. (T3.8) against (a a.) *E. coli*, (b.) *B. subtilis*, (c.) *Coliform* bacteria group, (d.) *S. cerevisiae* yeast on the first day of incubation with the description: 1. Microbial opponant, 2. clear zone, 3. isolates fungus *Exophiala* sp.

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