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Efficiency of Nitrogen Fertilization in Shallots *(Allium ascalonicum,.L)* with the Addition of Cow Manure

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ABSTRACT

This research was accomplished to establish the influence of nitrogen fertilizer and cow manure on the growth, yield, and efficiency of shallot plants growth. This study was accomplished in the rice fields of Brebes, Brebes Regency. This study is a factorial experiment using a randomized block design (RAK) with two factors. The first factor is the application of nitrogen fertilizer 2/3 N (ZA) + 1/3 N (Urea) which consists of 4 levels, there are 0 N kg/ha (N0); 100 N kg/ha (N_1); 200 N kg/ha (N_2) and 300 N kg/ha (N_3). The second factor is the application of organic material cow manure with 4 levels there are 0 t/ha (K_0); 10 t/ha (K_1); 20 t/ha (K_2) and 30 t/ha (K_3). There were 16 treatments, each action was repeated three times. Plant height, number of leaves, leaf area (g), number of tillers per plant, number of tubers per plant, fresh plant weight per clump (g), tuber weight per clump (g), dry tuber weight per clump (g), dry weight plants per clump (g), tuber volume per clump (mL), N uptake (g), fertilization efficiency N (%). Observational data were analyzed with the F test to establish diversity and if there were significant difference it was continued with Ducan's test with an error rate of 5%. The findings exposed that the administration of nitrogen dose of 200 kg N/ha increased growth and the yield of shallots was the best compared to other treatments. The best efficiency of N fertilization in the application of nitrogen fertilizer 100 kg N/ha.
Keywords: shallot, nitrogen, dose, efficiency, fertilizer

ABSTRAK

Kakao merupakan tanaman komoditas penting di Indonesia. Namun kendala yang ada saat ini terkait dengan masalah serasah kulit kakao yang lambat terurai menjadi kendala dalam pengelolaan perkebunan. Oleh karena itu perlu dilakukan penelitian terkait eksplorasi dan identifikasi jamur saprofit pada serasah buah kakao. Sampel serasah buah kakao diambil di PTPN XII, sedangkan proses isolasi dan identifikasi dilakukan di Program Studi Agroekoteknologi Universitas Brawijaya. Dari hasil identifikasi makroskopis dan mikroskopis diperoleh sembilan isolat jamur saprofit yang terdiri dari tujuh genus yaitu Candida sp., Fusidium sp., Penicillium sp., Chepalosporium sp., Rhizopus sp., Trichoderma sp.1, Aspergillus sp., Trichoderma sp.2, dan Trichoderma sp. 3. Hasil penelitian menunjukkan bahwa jamur pada serasah kulit kakao memiliki bentuk morfologi dan mikroskopis yang berbeda-beda.
Kata kunci: Pengendalian hayati, Busuk Buah Kakao, Dekomposer, Phytophthora palmivora

1.Introduction

Indonesia is one of the largest cocoa-producing countries in the world. Cocoa plantations are spread across almost all the islands of Indonesia with the largest producer on the island of Sulawesi. However, currently, there are several obstacles in cocoa cultivation. Whereas agricultural waste can be used as organic fertilizer, organic mulch and biological control materials (Basuki et al., 2022). One such obstacle is cocoa pod rot disease caused Phytophthora palmivora (Sukmadewi and Nikmah, 2022). Infected cocoa pod skin is a source of inoculum in the spread of the disease.

Damage by P. palmivora can vary from mild to moderate until the fruit cannot be harvested (Sriwati et al., 2019). It infects many parts of the cocoa plant including the pods, causing black pod rot disease (Walker, 2020). Severe damage if this fungus enters the fruit and causes rotting of the seeds. If it attacks the fruit nipples, it causes the fruit to mummify while the attack on the young fruit causes the growth of the seeds to be disrupted, namely, they become soft and greenish- brown in color, which results in a decrease in the quality of the seeds. Attacks on almost-ripe fruit have no significant effect on seed growth, but soft seeds occur and eventually, a decrease in seed odor is not good (Ditjenbun, 2011).
In Indonesia, this disease caused by P. palmivora can cause significant losses, especially in areas with wet climates. In Central Java losses can reach 49.8%, in East Java 52.99%, in West Java 42.30%, and in Sulawesi 15%. Although this pathogen attacks all parts of the plant, the greatest damage is caused by fruit rot, stem canker, and wilting of seedlings. Yield losses due to pod rot, stem cancer, and wilting of seedlings can reach 39%. In Ghana fruit loss due to P. palmivora ranges from 60-100% and as a result many farmers neglect their crops (Ramlan, 2010). In East Java losses can reach 52.99% (Sukamto, 2003). This can be exacerbated if the pathogen is already present in the endemic area (Tanzil et al., 2022)

Use of biocontrol agents against soil borne pathogen is gaining importance in the present situation for eco-friendly management of soil borne diseases (Das and Mahapatra, 2023). Exploration of unicellular microbes can be used as biological control agents (Tanzil et al., 2020). The role of antagonism that can be used as a biofungicide for controlling cacao fruit rot is Trichoderma sp (Widiyatmoko et al., 2019). Therefore it is necessary to carry out research related to the exploration, isolation, and identification of saprophytic fungi in cocoa pod litter in order to determine their role and potential in controlling cocoa pod rot disease.

2. Materials And Method

2.1 Area study and materials

Research conducted at Agroecotechnology Study Program, Brawijaya University. The material used isolated a collection of saprophytic fungi from cocoa peel litter from research (Efendi, 2014), potato dextrose agar (PDA) media, 70% alcohol, and sterile distilled water. The tools used were the knife, autoclave, scalpel, microscope, Bunsen burner, inoculating needle, aluminum foil, tissue, plastic wrapping, chlorox, chloram penicol, Erlenmeyer tube, scales, measuring cup, petri dish, electric stove, cover glass, object glass and laminar flow cabinets, large plastic bags, label paper, pens.

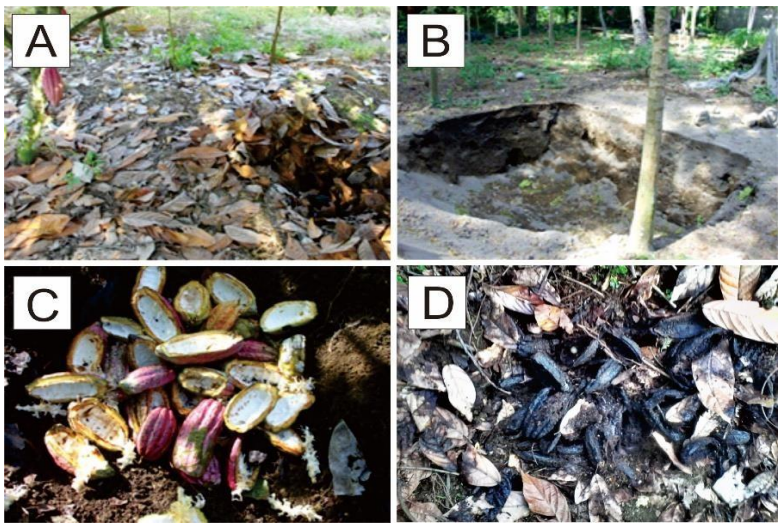

Figure 1. Cacao shell drain holes inside the garden (A), cocoa shell drain holes outside the garden (B), harvested cocoa shells (C), and cocoa shell litter on the land (D)

2.2. Isolation of saprophytic fungi.

The litter of cocoa pod shells that are almost decayed is taken from the cocoa shell immersion area at PT. Perkebunan Nusantara XII, Bantaran Gardens, Penataran Afdeling (Figure 1) which has a high intensity of the attack by P. palmivora so that the saprophytic fungi obtained later are fungi that are decomposers and biological agents. The sampling of cocoa shell litter was taken in one hectare at five places. Determining the place using a systematic method of diagonal sampling in order to obtain five places. At each location, three samples of cocoa shell litter were taken. The cocoa pod husk litter that has been obtained is washed and then cut into pieces of approximately 1 cm2. Then the pieces were sterilized using 2% chlorox solution once, 70% alcohol once, and distilled water twice, each time for 1 minute. After that, drain on dry tissue. Then, the specimen pieces were planted on PDA media (Efendi, 2014).

2.3. Fungi identification

A pure culture that has been purified, and incubated for 7 days. After that, a small number of fungi mycelia was taken to be placed on a glass preparation containing PDA media for saprophytic fungi, then the fungi isolates were covered with a covered glass and incubated for 3-4 days so that the spores of P. palmivora and saprophytic fungi could be seen. After incubation, observations were made using a microscope. Observations were made on the shape of the mycelia and the shape of the fungal spores. Then compared with Barnett's fungi identification book (1960).

3. Result And Discussion

3.1 Isolation and Identification of Saprophytic Fungus from Cocoa Cooler Litter

From 10 petri dishes as a result of exploration, the macroscopic appearance of the Fungus growing on PDA media was that the average colony was white, reddish-white, and had a rough texture. The speed of fungi growth also varied, some were up to the seventh day after inoculation, the fungus that grew was unable to fill the petri dish and some were on the third day after inoculation, the fungus that grew was able to fill the petri dish. The results are shown in Figure 2.

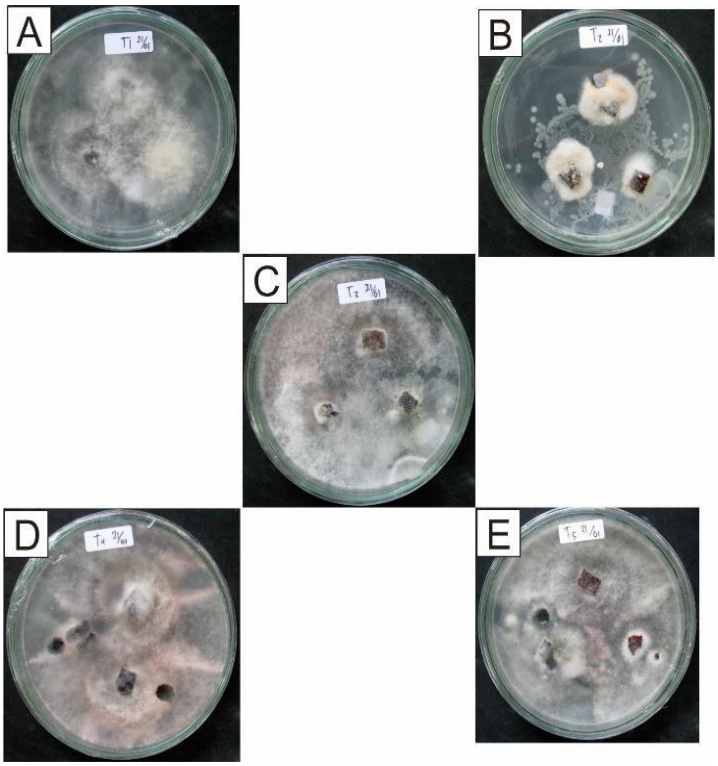

Figure 2. Isolation of saprophytic fungi from cocoa shell litter on PDA media. (A) Isolation from 1st place sample, (B) Isolation from 2nd place sample, (C) Isolation from 3rd place sample, (D) Isolation from 4th place sample, (E) Isolation from 5th place sample

The process of selecting and observing fungi colonies in Figure 2. for purification purposes is very difficult

because the macroscopic appearance of the fungus is almost the same and there are no clear boundaries between one fungi colony and another fungi colony. From the results of observations based on the dominance and appearance of the color of the colonies, the pattern of distribution, and the colors behind the petri dishes which were macroscopically different, 9 fungal isolates were obtained.

Table 1. Saprophytic fungi were found from cocoa husk litter samples

Location	Genus
Place 1	*Candida* sp. *Penicillium* sp. *Fusidium* sp.
Place 2	*Rhizopus* sp. *Chepalosporium* sp.
Place 3	*Trichoderma* sp.1
Place 4	*Trichoderma* sp.2
Place 5	*Aspergillus* sp. *Trichoderma* sp.3

Based on Table 1, in place 1, 3 genera of fungi were found, namely Candida sp., Penicillium sp., and Fusidium sp., in place 2, 2 genera of fungi were found, namely Rhizopus sp. and Cephalosporium sp. While in places 3 and 4, each genus was obtained, namely, Trichoderma sp.1 and Trichoderma sp.2. From place 5, 2 genera of fungus were obtained, namely, Aspergillus sp. and Trichoderma sp.3. The morphology and characteristics of the fungus macroscopically and microscopically are as follows:

3.2 Candida sp.

Macroscopic. From the results of macroscopic observations made, the fungi colonies were white and the color behind the colonies was also white, but in the middle, there was a black color that resembled a net. After 2 weeks the colony color changed to grayish black. Coarse colony texture is characterized by the growth of fungi mycelia that touch the top lid of the petri dish. The distribution pattern of the Fungus is regular but there are no concentric circles. Fungi growth is classified as very fast because the fungus takes 3 days to be able to fill the petri dish.

Microscopy. Based on the results of observations made using a microscope, the microscopic appearance of the fungus is shown in Figure 1. It can be seen that the fungal hyphae are hyaline, insulated, and have conidiophores that are not too long. At the end of the conidiophore, some conidia are slightly oval resembling peanut pods, that is, there is an indentation in the middle of the conidia. Based on the measurement results, the conidia reached 6.15 µm in length and 2.07 µm in width. The characteristics of these fungi are following those proposed by Barnett and Hunter (1960), who said that the microscopic characteristics of Candida sp., The fungi mycelium is insulated. Partially covered with conidia. Conidia hyaline, 1-celled, spherical to oval in shape, forming short chains with buds. Produced apically or laterally on the mycelium. Some are generally saprophytic and are often considered to be members of the yeast family.

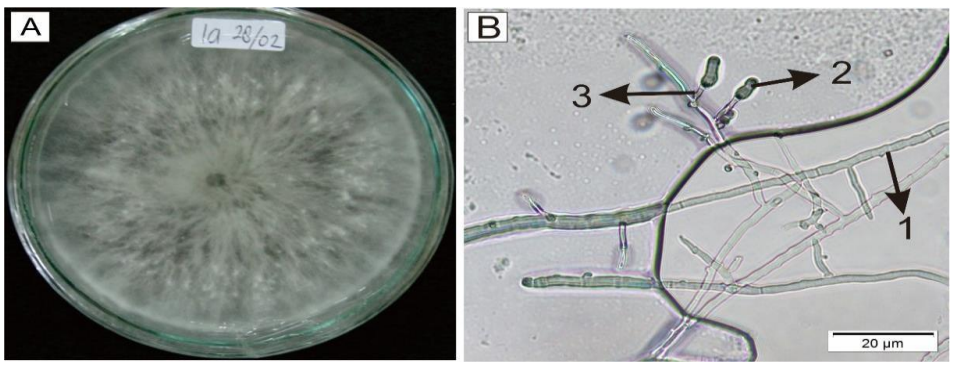

Figure 3. Macroscopic (A) and microscopic (B) appearance of pure cultures of the fungus Candida sp. 7 hsi on PDA media. B. hyphae (1), conidia (2), and conidiophores (3)

3.3. Penicillium sp.

Macroscopic. Based on the results of macroscopic observations that have been carried out, the following data is obtained, the fungi colonies are grayish- yellow, either on the surface or behind the petri dish. The texture of the fungi colonies is rough and the shape of the colonies is round with irregular edges of the colonies so that they have an irregular distribution pattern. There are no concentric circles in the fungi colonies but the growth of the fungus can be said to be quite slow because within 7 days the new fungus is able to fill the petri dish. These macroscopic characteristics are in accordance with the characteristics stated by Samson et al. (1981), the growth of mold colonies belonging to the genus Penicillium was slow, some even reached 52 mm after ten days of incubation. Colonies are flat, with a powdery coating, yellowish-green surface, and white margins.

Microscopy. The results of microscopic observations carried out using a microscope showed elongated hyphae and hyaline. Single conidiophore, upright, and at the end there are fields. Conidia chain at the end of fialids, 1-celled, round and hyaline. Conidia are arranged like chains that are interconnected between conidia to one. Barnett and Hunter (1960) stated that fungi have the characteristics of a single or branched conidiophore, at the end of which there are several fialids, and hyaline or bright conidia, in the form of 1 cell and arranged in a chain. Based on macroscopic and microscopic descriptions, the fungus is Penicillium sp.

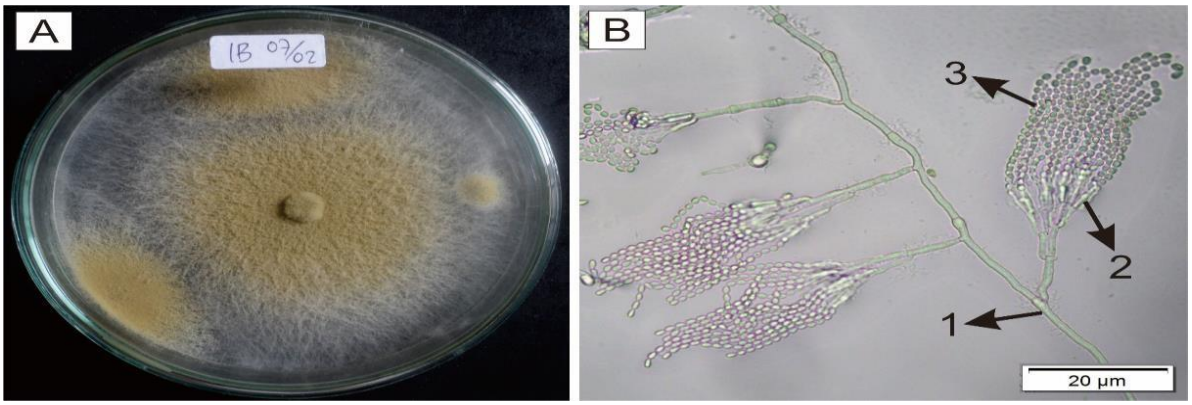

Figure 4. Macroscopic (A) and microscopic (B) appearance of the pure culture of the fungus Penicillium sp. 8 days old on PDA media. B. hyphae (1), fialids (2), and conidia (3)

3.4. Fusidium sp.

Macroscopic. Based on the results of macroscopic observations that have been made, the following data is obtained, the fungi colonies are grayish- white but in the middle, the color of the colonies tends to be grayish-black. Almost the same as the surface color of the colony, the back color of the colony is also grayish with a black color in the middle. The texture of the fungi colony is rather rough and has an irregular distribution pattern. In the fungi colonies, there are no concentric circles but the growth of the Fungus can be said to be fast because within three days the fungus can fill the petri dish**.**

Microscopy. Based on the microscopic observations made, the hyphae of hyaline Fungi have partitions, and branches and have conidiophores at the ends of which some conidia are oval or elliptical in shape. Conidia are arranged like a chain, connecting one conidium to another. From the measurement results, the conidia have a length of approximately 4.71 µm and a width of 3.82 µm. The characteristics of the microscopic fungi are following those proposed by Barnett and Hunter (1960), conidiophores are usually short and have simple branches. Conidia are hyaline, single-celled, oval, and usually arranged into conidia chains. This fungus can be saprophytic and parasitic.

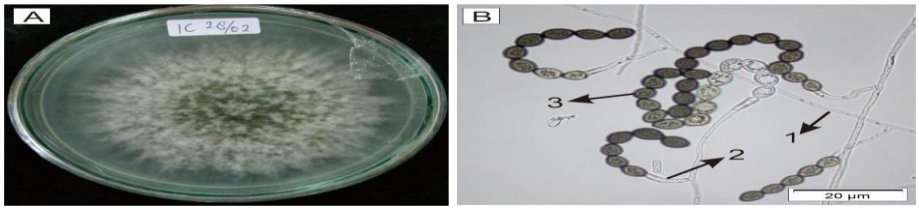

Figure 5. Macroscopic (A) and microscopic (B) appearance of the pure culture of the fungus Fusidium sp. 8 days old on PDA media. B. hyphae (1), conidiophore (2), and conidia (3)

3.5. Rhizopus sp.

Macroscopic. From the results of macroscopic observations, the following data were obtained, at the beginning of growth, the fungi colonies were white and resembled fibers. However, after a few days, there were black spots on the colonies. These black spots are spores attached to the top of the hyphae like small blackish-brown grains. The texture of the fungi colony is rather rough and thick. While the distribution pattern of the fungus is irregular. Fungal colonies do not have concentric circles. The growth of the Fungus can be said to be quite fast because, within six days, the fungus was able to fill the petri dish.

Microscopy. From observations using a microscope, it can be seen that the fungus has hyphae and conidiophores which are hyaline and not insulated. At the upright end of the conidiophore, there is a black ascus. The ascus is the site where spores are produced. From the measurement results, the ascus has a width of approximately 14.25 µm and a length of 10.22 µm. These microscopic characteristics are following what Postlethwait and Hopson (2006) stated, that the fungus Rhizopus sp. has chlamydospores globose, elliptical, or cylindrical with a size of 7-30 µm or 12-45 x 7-35 µm. Rhizopus sp. has a characteristic that has hyphae that form rhizoids to attach to the substrate. Another characteristic is that it has coenocytic hyphae, so it is not septate or insulated. Mycelium from Rhizopus sp. which are also called stolons spread over the substrate due to the activity of vegetative hyphae. Rhizopus sp. reproduces asexually by producing many stalked sporangiophores. This sporangiophore grows upward and contains hundreds of spores. Sporagiophore is separated from other hyphae by a septa-like wall. One example of this species is Rhizopus stolonifer which usually grows on stale bread. Based on macroscopic and microscopic descriptions, the fungus is Rhizopus sp.

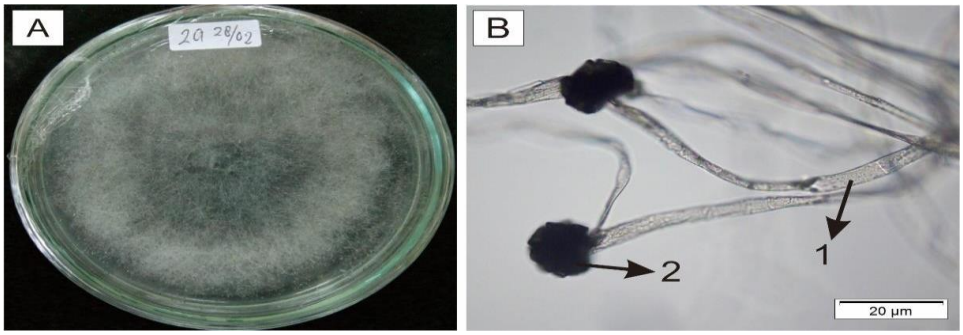

Figure 6. Macroscopic (A) and microscopic (B) appearance of the pure culture of the fungus Rhizopus sp. 8 days old on PDA media, (B) conidiophores (1), ascus (2)

3.6. Cephalosporium sp.

Macroscopic. The macroscopic characteristics of the fungus are as follows, white fungi colonies, both on the surface and behind the petri dish. The texture of the fungi colonies is smooth and has a regular distribution pattern. In the fungi colonies, there are concentric circles but the growth of the Fungus can be said to be very slow because, within 7 hsi, the Fungus is only 5.5 cm in diameter. These macroscopic characteristics are following the characteristics presented by Gangjar et al. (1999), who stated that the appearance of the colonies was like cotton and white to pink in color. The colony growth rate is very slow. In the middle, it looks like cotton.

Microscopy. Microscopic observations show that the hyphae are long and slit. As well as having a very simple branching. Conidiophores are hyaline, unbranched, insulated, and quite long. The conidia at the ends of the conidiophores are clustered, hyaline, and oval. According to Barnett and Hunter (1960), Cephalosporium has slender conidiophores or simple swells, hyaline conidia consisting of 1 cell and forming at the end of the conidiophores, and conidia in groups. Based on the macroscopic and microscopic descriptions of this fungus, Cephalosporium sp.

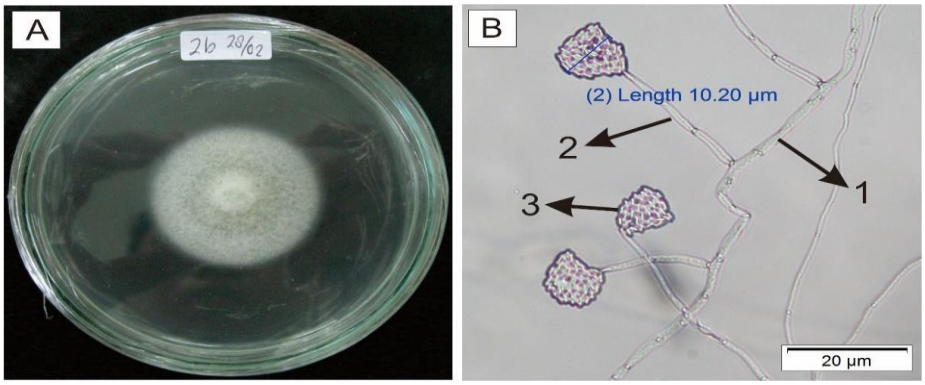

Figure 7. Macroscopic (A) and microscopic (B) appearance of pure culture of the fungus Cephalosporium sp. age 8 hsi on PDA media, B. hyphae (1), conidiophores (2), and conidia (3)

3.7. Trichoderma sp. 1

Macroscopic. Based on the results of macroscopic observations that have been carried out, the following data is obtained, at the beginning of the growth of the fungi colony it is very thin and has a clear green color. However, after a few days, the fungi colonies are yellowish- green with white edges. The texture of the fungi colonies is soft and has a regular distribution pattern. In the fungi colonies, there are no concentric circles but the growth of the Fungus can be said to be fast because within three days the fungus can fill the petri dish. According to Rifai (1969), things that can affect the thickness of a fungi colony on artificial media are the nature of the fungus itself, the nutrient content of the media, temperature, light, and even the thickness of the media can affect growth and shape of the fungus colonies.

Microscopy. Based on observations using a microscope, fungi have hyaline, insulated, and branched hyphae. In addition to hyphae, conidiophores are also visible, which are hyaline, insulated, and branched. Fungi conidia appear dark green, and round in shape, and appear to be arranged in clusters at the ends of the fialids. The macroscopic and microscopic characteristics of the observation results are in line with those proposed by Gandjar et al. (1999), Colonies on Oat Agar medium reached a diameter of more than 5 cm within 9 days, initially hyaline in color, then turned greenish-white and then dim green, especially in parts that showed lots of conidia. Otherwise, the colonies are colorless. Conidiophores can branch like a pyramid, namely at the bottom of the lateral branches that are repeated, while towards the ends of the branches they become shorter. The fialids are slender and elongated, especially on the aspect of the branches, and measure 18 x 2.5 µm. Conidia are semi-spherical to short oval, (2.8 - 3.2) x (2.5-2.8) µm in size, and smooth-walled. Chlamydospores are generally found in the mycelia of old colonies, are intercalated and sometimes terminal, generally spherical, hyaline in color, and smooth-walled. Based on macroscopic and microscopic descriptions, this fungus is Trichoderma sp.1.

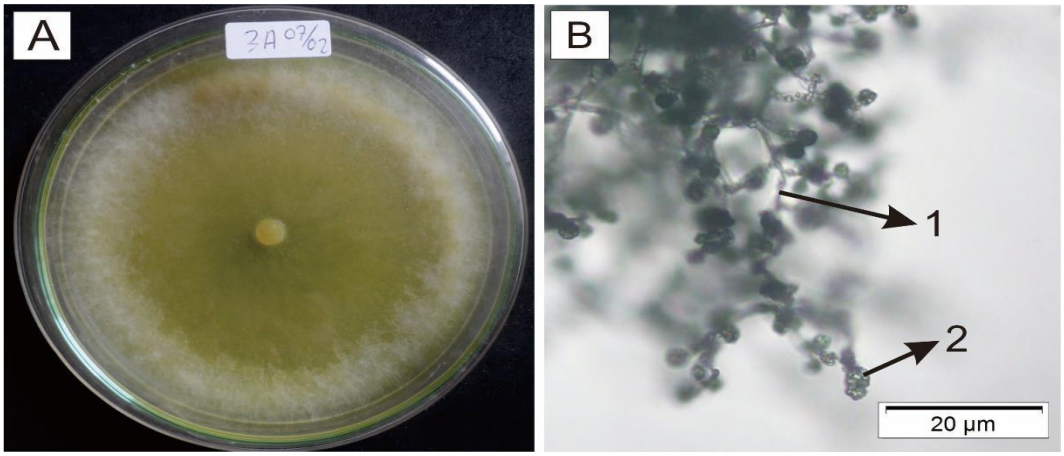

Figure 8. Macroscopic (A) and microscopic (B) appearance of pure culture of the fungus Trichoderma sp.1 aged 7 hsi on PDA media, B. conidiophores (1) and conidia (2

3.8. Trichoderma sp. 2

Macroscopic. Based on the results of observations, the fungi colonies were greenish blue and the color behind the colonies was faded blue. Concentric circles are clearly visible on the colony so that the colony looks like a

mosquito coil. The texture of the fungi colonies is rough and has an irregular distribution pattern. Fungi growth can be said to be fast because within four days the fungus is able to fill the petri dish. These macroscopic characteristics are in accordance with the characteristics presented by Rifai (1969). Macroscopically, the fungus Trichoderma spp. can be distinguished by the speed of growth in a petri dish. This fungus can grow quickly in 5 days at a temperature of 25 0C. Most members of the genus Trichoderma spp. form colonies that have different colors and form colonies with circular zones that are visible in the light.

Microscopy. Based on observations using a microscope, fungi have hyaline, insulated, and branched hyphae. In addition to hyphae, conidiophores are also visible, which are hyaline, insulated, and branched. Fungi conidia appear dark green, round in shape, and appear to be arranged in fialids. These characteristics are in accordance with the characteristics stated by Domsch et al. (1980), Trichoderma spp. belongs to the class Deuteromycetes, order Moniliales, family Moniliaceae. Trichoderma spp. having smooth-walled conidia, the colony is initially hyaline in color, then it becomes greenish-white, and then dark green, especially in parts that show a lot of conidia. Conidiophores can branch like a pyramid, namely, at the bottom the lateral branches are repeated while getting to the end of the branching becomes shorter. The phialids are slender and elongated, especially at the apex of the branches. Conidia are semi-spherical to short oval. Based on macroscopic and microscopic descriptions, this fungus is Trichoderma sp.2. Phialides were found constricted at the base, more or less swollen near the middle and abruptly near the apex into short subcylindric (Anees et al., 2018) neck

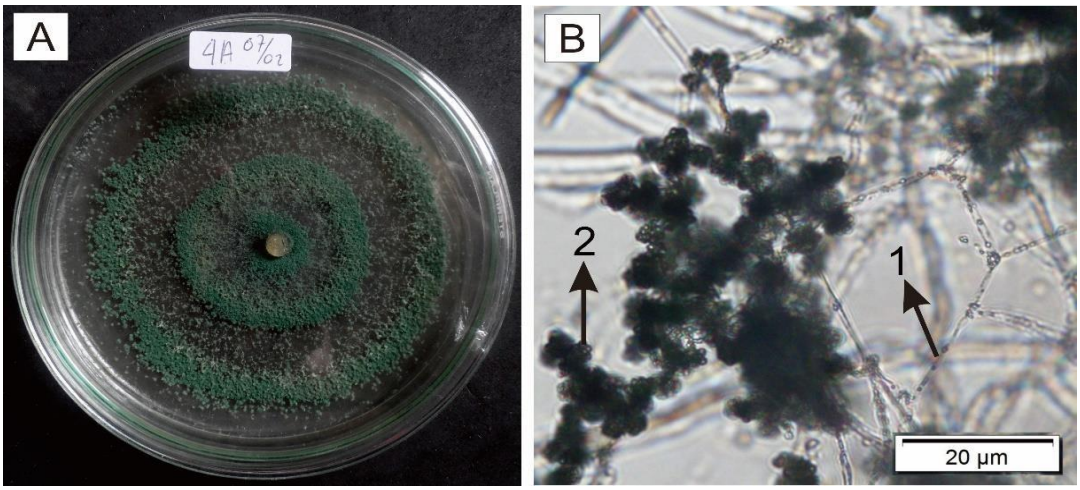

Figure 9. Macroscopic (A) and microscopic (B) appearance of pure culture of the fungus Trichoderma sp.2 aged 7 hsi on PDA media, B. conidiophores (1) and conidia (2)

3.9. Aspergillus sp.

Macroscopic. Based on the results of macroscopic observations, the growth of the Fungi colonies was round, and the middle part of the colony was black caused by piles of spores, with flat edges and translucent white. The color of the colony behind the petri dish is yellowish-white. At the beginning of the growth the colony is thin and smooth white, after a few days the colony turns black and thickens like cotton. Colony growth spreads unevenly like flour grains. On the eighth day of the incubation period, the diameter is 4.7 cm.

Microscopy. The results of microscopic observations showed fungal hyphae elongated, not insulated, and hyaline. Conidiophores are erect, unbranched, and hyaline. At the end of the conidiophore, there are conidia that collect black. Barnett and Hunter (1960), described that the microscopic characteristics of the fungus Aspergillus sp. has a simple upright conidiophore, ends with a swollen tip and the conidia consist of 1 cell and are black. In addition, these microscopic characteristics are also in accordance with those stated by Gandjar (1999), Aspergillus conidiophores end in a fan vesicle. The stalks of conidiophores are hyaline and are generally thick-walled and conspicuous. The heads of the conidia are round, then split into separate columns. The vesicles are spherical to semi- spherical, and 25–50 µm in diameter. Fialids are formed directly on the vesicles or on the metula, i.e. on the large heads of conidia, and are (10-15) x (4-8) µm in size. Metula is (7-10) x(4- 6) µm in size. Conidia are spherical to semi-spherical, 5-6.5 µm in diameter, and yellow-brown in color. Habitat is very common in the tropics and is found in soil, litter, spices, maize and cereals. Based on macroscopic and microscopic descriptions, this fungus is Aspergillus sp.

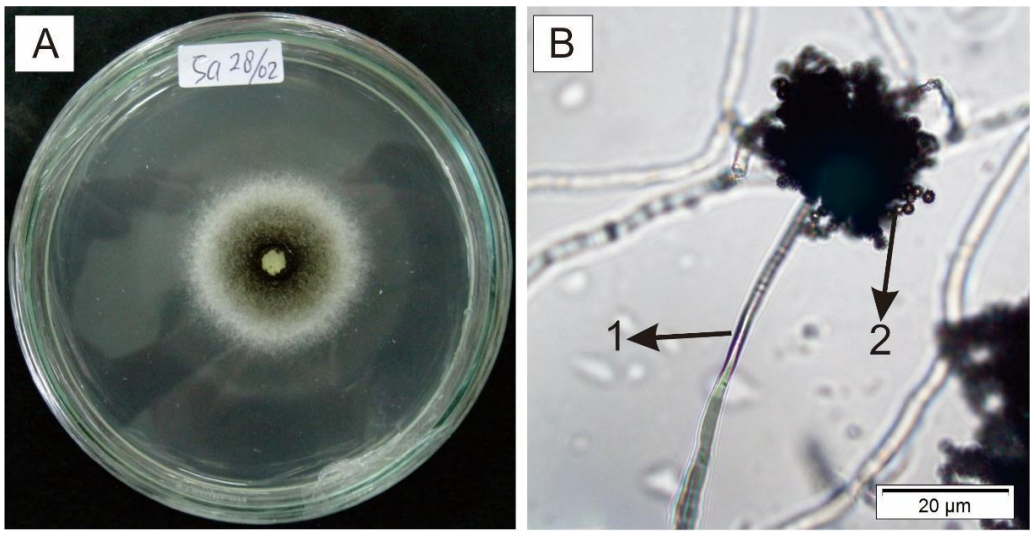

Figure 10. Macroscopic (A) and microscopic (B) appearance of the pure culture of the fungus Aspergillus sp. 8 days old on PDA media, B. conidiophores (1), and conidia (2)

3.10. Trichoderma sp. 3

Macroscopic. Based on the results of macroscopic observations that have been carried out, the following data is obtained, the fungi colonies are whitish green and in the middle, there is a blue color. The texture of the fungi colonies is rough and has an irregular distribution pattern. In the fungi colonies, there are no concentric circles but the growth of the fungi s can be said to be quite fast because within four days the fungus can fill the petri dishes.

Microscopy. From the results of microscopic observations carried out using a microscope, the following characteristics were obtained, the fungal hyphae appear hyaline, and insulated. Conidiophores grow from hyphae and it appears that at the ends of the conidiophores, there are black lumps, which are collections of conidia. Microscopic features of Trichoderma sp. This is in accordance with the microscopic characteristics proposed by Gandjar et al. (1999), conidiophores of Trichoderma sp. can branch like a pyramid, namely at the bottom of the lateral branches, branching towards the ends becomes shorter. Fialid looks slim and long measuring 18 x 2.5 µm. Conidia are semi-spherical to short oval, (2.8-3.2) x (2.5-2.8) µm in size, and smooth-walled. Based on the macroscopic and microscopic descriptions that have been done, this fungus is Trichoderma sp.3. Trichoderma are prominent with their conidiophores with dense masses of conidia, such as tufts, and short branches growing at 90° or 100° from the conidiophore; they have from one to four phialides, long, irregular and single-celled, bottle-shaped, with small irregular branches arising from the center of the vesicle, with conidia at the apex, globose, subglobose or ellipsoidal (Mendez et al., 2020).

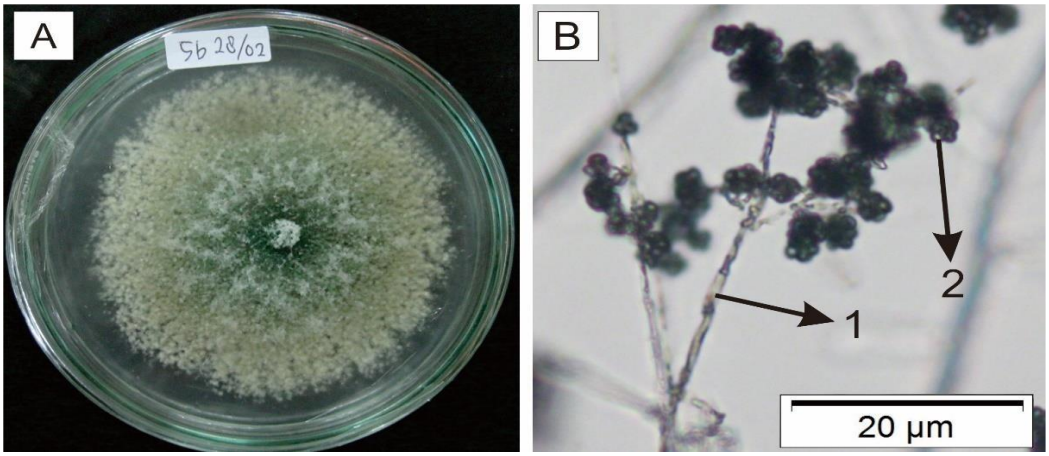

Figure 31. Macroscopic (A) and microscopic (B) appearance of pure culture of the fungus Trichoderma sp.3 aged 7 hsi on PDA media, B. conidiophores (1), and conidia (2)

4. Conclucion

This study concluded that nine isolates of saprophytic fungi were found from cocoa husk litter, namely, Candida sp., Penicillium sp., Fusidium sp., Rhizopus sp., Chepalosporium sp., Trichoderma sp.1, Trichoderma sp.2, Aspergillus sp., and Trichoderma sp. 3. Regarding the suggestion to carry out further research related to the benefits of saprophytic fungus in the field.

4.2. Suggestion

Further studies using additional agroclimatological elements and agronomic action characteristics are needed. In addition, the data used covers a larger time span, thus ensuring reliable analytical findings.

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