

## Effect of Organic and Biological Fungicides on the Development of *Ganoderma* Fruiting Bodies

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### ABSTRACT

*Ganoderma sp.* are major pathogens of oil palm (*Elaeis guineensis*), causing basal stem rot and severe yield losses. This study investigated the efficacy of an allicin-based organic fungicide and a *Trichoderma*-based biofungicide on the growth of *Ganoderma* mycelium in vitro and on fruiting body development in vivo. The in vitro experiment used PDA media amended with different concentrations (0–40%) of an emulsified concentrate (EC) organic fungicide, while the in vivo test evaluated treatments on naturally occurring *Ganoderma* fruiting bodies growing on dead breadfruit trunks. The organic fungicide was applied twice (three-day intervals), followed by one biofungicide application. The EC formulation completely inhibited mycelial growth at concentrations  $\geq 2\%$  (0.67% active ingredient). In field trials, fruiting body enlargement ceased within three weeks after treatment, while untreated controls continued to expand. At 3.5 months post-treatment, *Ganoderma* could no longer be isolated from treated fruiting bodies, whereas viable mycelia persisted in controls. Combined application of organic and biofungicides resulted in tissue browning, compact texture, and death of the fungus. These findings indicate that allicin-based organic fungicides, especially when followed by *Trichoderma* treatment, can effectively suppress *Ganoderma* fruiting body growth and viability. Further trials on living oil palm tissues are recommended to confirm field applicability.

Keywords: allicin, biofungicide, fruiting body inhibition, *Ganoderma sp.*, organic fungicide, *Trichoderma*

### INTRODUCTION

*Ganoderma* is a major pathogen of oil palm that causes significant losses in oil palm plantations. This fungus can also infect other economically valuable plants such as rubber, eucalyptus, and acacia (Mohammed *et al.* 2014). *Ganoderma boninense* is characterized by large, perennial, rough basidiocarps (fruiting bodies), which are sometimes stalked. The fruiting body typically has a fan or hoof

shape on the tree trunk, producing double-walled spores that are yellowish to brownish. In addition to being a soil-borne pathogen, this fungus is also a facultative parasite that can live as a saprobe on decaying stumps and roots, especially on suitable host plants (oil palm). Several control strategies against *Ganoderma* have been developed through improvements in technical, chemical, and biological methods. Environmentally friendly control of *Ganoderma* has become a choice for farmers in line with food safety require-

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ments that have become a consumer lifestyle today. Organic fungicides are increasingly being used to control various plant pathogens. Organic fungicides with the active ingredient allicin have been shown to effectively kill several pathogens (Sarfraz et al. 2020), such as damping-off disease in tomatoes. Research by An et al. (2022) showed that the addition of *Trichoderma reesei* to compost applied near the fruiting bodies of *Ganoderma* growing from trees was able to suppress the growth of *Ganoderma* sp. fruiting bodies, with an average surface area of 18.77 cm<sup>2</sup>. Meanwhile, the fruiting bodies of *Ganoderma* with compost treatment alone developed better, with an average surface area of 65.19 cm<sup>2</sup>, and the average surface area of the control *Ganoderma* fruiting bodies was 62.12 cm<sup>2</sup>. This indicates that the addition of *T. reesei* has a significant effect on inhibiting the growth of *Ganoderma* sp. fruiting bodies. This study aims to test the effectiveness of organic fungicides with the active ingredient allicin and biological fungicides against the development of *Ganoderma* fruiting bodies.

## MATERIALS AND METHODS

The *Ganoderma* isolates used in the *in vitro* test were obtained from oil palm plantations in Rejosari and Bekri, Lampung Province. The *Ganoderma* cultures were rejuvenated on potato dextrose agar (PDA) medium and incubated for 14 days at room temperature.

The organic fungicide was made from spices, with allicin as the active ingredient, derived from local Indonesian garlic. The garlic and water (1:2, v/v) were blended and filtered to obtain the filtrate. The filtrate was then formulated as an Emulsified Concentrate (EC) organic fungicide by adding 10% clove oil and surfactants at 10% and 2.5%, respectively. The biological fungicide was made from *Trichoderma* obtained from oil palm plantations in Medan, North Sumatra, and rejuvenated on PDA medium for 5 days. The *Trichoderma* sp. isolate from one Petri dish was blended

in 100 mL of water to obtain a mycelial suspension with a density of 10<sup>6</sup> CFU per mL.

### Test of EC Organic Fungicide Against *Ganoderma in vitro*

This test was conducted in two stages. In the first stage, agar poisoning was carried out by adding a certain amount of EC organic fungicide (according to treatment) to sterile PDA medium that was still liquid at around 60 °C, then pouring it into sterile Petri dishes. The EC concentrations used consisted of five levels: 0, 10, 20, 30, and 40%, each replicated twice. *Ganoderma* mycelial colonies were then inoculated in the center of the PDA medium in the Petri dish. The effectiveness of the EC fungicide was measured by the inhibition of *Ganoderma* mycelial growth after 5 days of incubation on PDA medium in a dark room.

The second stage aimed to more precisely determine the effective concentration of the EC organic fungicide tested. This test was carried out in the same way as the first stage, except that the EC concentrations tested in this stage consisted of 12 levels: 0, 0.5, 1.0, 1.4, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, and 15.0%, each replicated twice. These corresponded to 0; 0.17; 0.33; 0.47; 0.67; 1; 1.33; 1.67; 2; 2.67; 3.33; and 5% active ingredient source (AIS).

### Test of EC Organic Fungicide and Biological Fungicide Against *Ganoderma in vivo*

This test used two *Ganoderma* sp. fruiting bodies that grew naturally on dead breadfruit tree trunks. The fruiting body used as the control was located on the upper position, while the treated one was below it. The EC organic fungicide at a 20% concentration was evenly applied with a brush over the entire surface of the *Ganoderma* fruiting body on days 0, 50, 51, and 52. Then, from days 56 to 58, the *Trichoderma* biological fungicide was applied in the same way. Both the EC fungicide and the biofungicide were applied to the underside of the fruiting body (which

contains many pores) and the upper surface. The control fruiting body received neither EC nor biofungicide treatment.

On day 65, nutrient agar medium in a Petri dish was placed under the *Ganoderma* fruiting body for one minute, then incubated for 3 to 7 days. The microorganisms that grew on the nutrient agar medium were observed on day 7. On day 104, the *Ganoderma* fruiting body was cut at the base, and a 1 cm<sup>2</sup> piece was cultured on PDA medium to observe the viability of *Ganoderma*. The growth of *Ganoderma sp.* on breadfruit wood was continuously monitored until day 192.

## RESULTS AND DISCUSSION

The *in vitro* test of organic fungicide effectiveness using the emulsified concentrate (EC) formulation showed that at a 10% concentration, after 5 days of incubation, the *Ganoderma sp.* mycelium did not grow. In contrast, the *Ganoderma sp.* mycelium grown on PDA medium alone (control) reached a diameter of 3.5 cm on the 5th day and continued growing until the 14th day. The hyphal structure, which was initially white and hyaline, began to thicken—especially noticeable starting from the 10th day of incubation (Table 1). On the medium without organic fungicide, *Ganoderma sp.* mycelium was able to grow up to a diameter of 9 cm (Table 1). The subsequent stage of *in vitro* testing showed that at low doses of organic fungicide,

namely 0.5% (on the 10th day) to 1% EC (on the 14th day), mycelial growth inhibition occurred. At a concentration of 1.4% EC, the *Ganoderma sp.* mycelium from Bekri began to thicken starting from day 7. At this concentration, changes in the mycelial texture were observed—becoming thicker, denser, compact, hardened, and slightly darker in color. At a higher concentration, namely 2% EC or 0.67% active ingredient source (AIS), *Ganoderma sp.* mycelium did not grow at all during 14 days of incubation. The results were similar for *Ganoderma sp.* isolates from both Rejosari (Table 2) and Bekri (Table 3). However, the *Ganoderma sp.* from Rejosari appeared more sensitive than that from Bekri, as indicated by the wider mycelial growth of *Ganoderma sp.* Rejosari (3.5 cm) compared to *Ganoderma sp.* Bekri (2 cm). By the fifth day of incubation, both had reduced to 0.2 cm.

Figure 1 (left) shows *Ganoderma sp.* mycelial growth on PDA medium without treatment (control), while the middle image shows *Ganoderma sp.* growth on PDA medium treated with 10% organic fungicide after 14 days of incubation. To determine the effect of organic fungicide application on *Ganoderma* viability, the treated cultures were transferred to fresh PDA medium without organic fungicide. The right image shows that *Ganoderma sp.* was unable to regrow on the new PDA plate. These results indicate that, based on the *in vitro* test, the application of organic fungicide can kill *Ganoderma sp.*

Table 1 Diameter (cm) of *Ganoderma* Rejosari in the *in vitro* efficacy test of emulsified concentrated organic fungicide up to 14 days of incubation

Dose, %EC (v/v)	5 days	7 days	10 days	14 days
40	0	0	0	0
30	0	0	0	0
20	0	0	0	0
10	0	0	0	0
0	3,5	7	9,0	9,0

Table 2 Diameter (cm) of *Ganoderma* Rejosari in the *in vitro* efficacy test of crude organic fungicide extract up to 14 days of incubation

Dose (b/v)		Incubation (days)			
% EC	%SAI	5	7	10	14
15	5	0	0	0	0
10	3,33	0	0	0	0
8	2,67	0	0	0	0
6	2	0	0	0	0
5	1,67	0	0	0	0
4	1,33	0	0	0	0
3	1	0	0	0	0
2	0,67	0	0	0	0
1	0,47	0	0	0	thicken
1	0,33	0	0	0	0,2
0,5	0,17	0,2	thicken	0,2	4
0	0	3,5	7	7	7

EC: Emulsified concentrate

SBA : Source of active ingredient

Table 3 Diameter (cm) of *Ganoderma* Bekri in the *in vitro* efficacy test of crude garlic extract up to 14 days of incubation

Dose (b/v)		Incubation (days)			
% EC333	%SAI	5	7	10	14
15	5	0	0	0	0
10	3,33	0	0	0	0
8	2,67	0	0	0	0
6	2	0	0	0	0
5	1,67	0	0	0	0
4	1,33	0	0	0	0
3	1	0	0	0	0
2	0,67	0	0	0	0
1	0,47	0	thicken	thicken	thicken
1	0,33	0	0	0	0
0,5	0,17	0,2	2	3	9
0	0	2	7	7	7

EC: Emulsified concentrated

SAI: Source of active ingredient

In the *in vivo* test of the organic fungicide, on day zero, the size of the control fruiting body was smaller than that of the treated fruiting body. Observations showed that visually, there was no apparent effect of the organic fungicide on the development of the fruiting body in terms of color, structure, or size. However, after three weeks, growth inhibition was observed in the treated *Ganoderma sp.* fruiting body, resulting in both the control and treated fruiting bodies being of similar size. This result indicates that it takes approximately three weeks for the organic

fungicide to inhibit the growth of *Ganoderma sp.* fruiting bodies. The inhibition appeared to persist, and one month after the first stage of organic fungicide application, the size of the control fruiting body was significantly larger than that of the treated one. These findings show that the organic fungicide strongly inhibits the growth of *Ganoderma sp.* fruiting bodies. This condition continued until day 49 (July 29) (Figure 1). The treatment was continued with a second application of organic fungicide for three consecutive days. This application

occurred 50 days after the first application. The organic fungicide was applied to both the top (cap) and bottom (lamella) of the plant. It was observed that the organic fungicide was absorbed into the tissue of the *Ganoderma sp.* fruiting body, although some of it might have evaporated (Figure 3). The control fruiting body continued to grow, increasing in size, whereas the fruiting body treated with the organic fungicide did not grow. There was a change in the texture of the treated fruiting body it became compact, dense, and dark in color. The edge of the treated fruiting body turned slightly brown, while the control fruiting body remained bright white (Figure 4).

At 56 days after the first organic fungicide treatment, the fruiting body was treated with *Trichoderma sp.* by applying it to both the upper (cap) and lower (lamella) parts of the fruiting body. Visual observation showed that the treated fruiting body became darker brown with a more compact texture, while the control fruiting body continued to enlarge and its edges remained bright white (Figure 5). The control fruiting body continued to grow until day 60, while the fruiting body treated with the first stage of organic fungicide did not. Observation of color also showed changes, where the treated fruiting body appeared browner with a denser texture (Figure 6). On the 65<sup>th</sup> day after the first application of organic fungicide, bacterial growth was observed from the fruiting bodies. Incubation results showed bacterial growth in the control fruiting body, but no bacterial growth from the treated one (Figure 7). These results suggest that

the application of the organic fungicide likely killed *Ganoderma sp.* as well as the bacteria growing on the fruiting body.

Observations were continued up to 101 days after the first application of organic fungicide. Growth inhibition of the treated fruiting body was still evident, while the control fruiting body continued to grow. Both color and texture still showed similar results as in previous observations (Figure 8). At 104 days, the fruiting bodies were broken apart, and *Ganoderma sp.* isolation was conducted using PDA medium. The incubation results showed that *Ganoderma sp.* could be isolated from the control fruiting body. In contrast, *Ganoderma sp.* did not grow from the treated fruiting body. However, *Trichoderma sp.* was successfully isolated from the treated fruiting body. An antagonism test between the isolated *Trichoderma sp.* and *Ganoderma sp.* (from the control) showed balanced growth between the two organisms (Figure 9). Observation of the fruiting body development continued at the site where they originally grew. The results showed that 26 days after the fruiting body was broken, new primordia (young fruiting bodies) developed at the control site, while no fruiting body growth occurred at the treated site (Figure 10). The fruiting bodies at the control site continued to develop until 87 days after the fruiting body breakage. The growth rate of the control fruiting bodies ranged from 0.04 cm to 0.2 cm, although between days 79 and 87 of incubation, a decline in growth rate occurred, with an average rate of 0.03 cm per day (Figure 10).

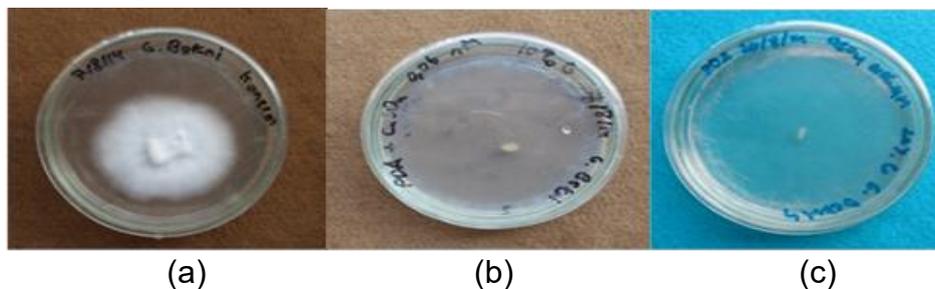


Figure 1 *Ganoderma* colonies grown on ADK medium (a) and *Ganoderma* grown on ADK medium + 10% organic fungicide formula (EC) (b) and retesting of *Ganoderma* treated with organic fungicide on new ADK medium (c).



Figure 2 Development of *Ganoderma* fruit bodies of control (top) and those treated with organic fungicide (bottom) a. on day 19, b. day 0, and c. day 49 after the first stage of FO application

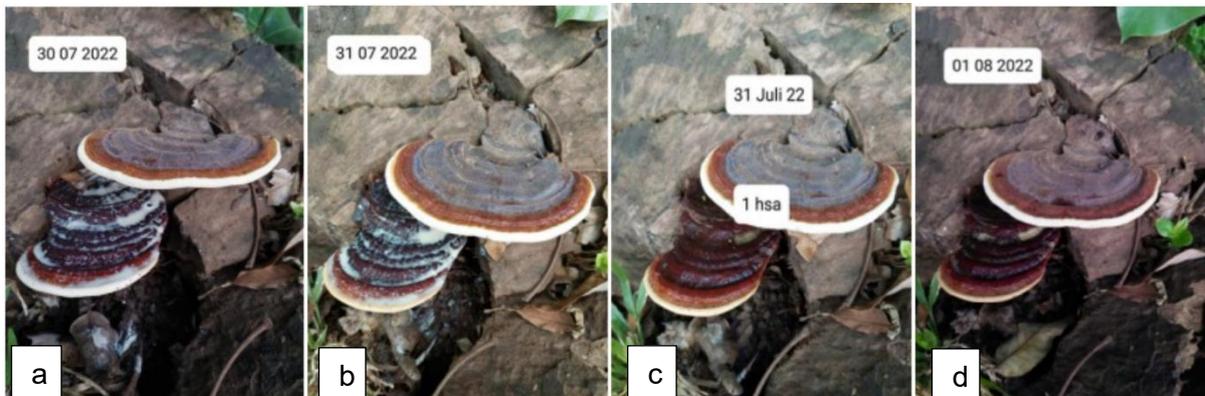


Figure 3 Development of *Ganoderma* fruit bodies of control (top) and those treated with organic fungicide (bottom) stage II consecutively for 3 days. a. On the 50<sup>th</sup> day, b. on the 51<sup>st</sup> day, c. on the 51<sup>st</sup> day (3 hours after FO application), d. on the 52<sup>nd</sup> day after FO application stage 1



Figure 4 Development of control (top) and treated (bottom) *Ganoderma* fruit bodies. a. on day 53<sup>rd</sup>, b. day 54<sup>th</sup>, and c. 55<sup>th</sup> day after FO application stage 1



Figure 5 Administration of *Trichoderma sp.* on treated *Ganoderma* fruit bodies (bottom) for 3 consecutive days. The fruit body of the treatment appears browner with a harder or more compact structure. a. On the 54<sup>th</sup> day seen from above, b. day 54<sup>th</sup> seen from below, c. day 55<sup>th</sup> seen from below, d. day 55<sup>th</sup> seen from above and e. 56<sup>th</sup> day after FO application stage 1.



Figure 6 Development of *Ganoderma* fruit bodies of control (top) and treatment (bottom) from incubation a. On day 57<sup>th</sup>, b. day 58<sup>th</sup>, c. day 59<sup>th</sup>, d. day 60<sup>th</sup>, e. day 61<sup>st</sup>, and f. day 62<sup>nd</sup> days after application of organic fungicide stage 1.

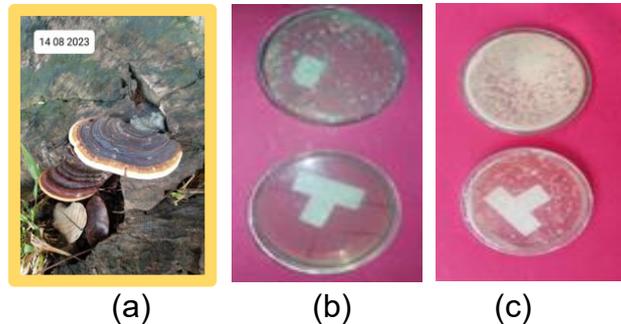


Figure 7 *Ganoderma* fruiting bodies 63 days after application of organic fungicide I (a), bacterial colonies from control (top) and treatment (bottom) *Ganoderma* fruiting bodies. 3-day incubation (b) and 1-week incubation (c).

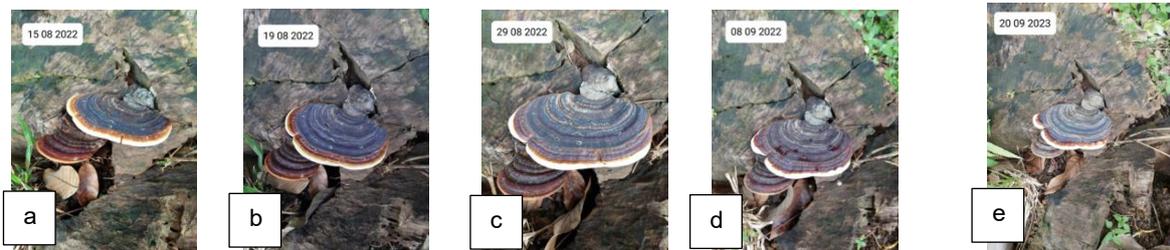


Figure 8 Development of control (top) and treated (bottom) *Ganoderma* fruiting bodies a. on day 64<sup>th</sup>, b. day 69<sup>th</sup> c. day 79<sup>th</sup>, d. day 89<sup>th</sup>, and e. 100<sup>th</sup> day after FO application stage 1

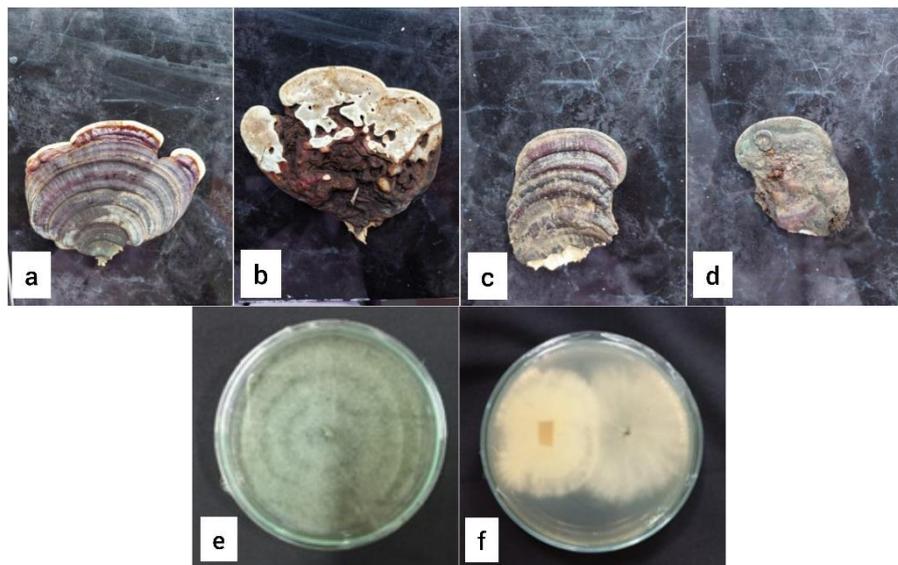


Figure 9 Control fruit body upper part (a), lower part (b), treatment fruit body upper part (c) lower part (d) Trichoderma isolated from treatment *Ganoderma* fruit body (e) and *Ganoderma* vs *Trichoderma* antagonist test (f).



Figure 10 Development of *Ganoderma* sp fruiting bodies after breaking, *Ganoderma* control fruiting bodies (top) and treatment (bottom) a. On the 26<sup>th</sup> day, b. 33<sup>rd</sup> day, c. 36<sup>th</sup> day, d. 45<sup>th</sup> day, e. 52<sup>nd</sup> day, and f. 60<sup>th</sup> day after breaking.

Observations from the *in vitro* test showed that the organic fungicide affected the growth of *Ganoderma* sp. mycelium. It is suspected that the active compound contained in the organic fungicide, namely allicin (Widiastuti et al. 2016), inhibited the development of *Ganoderma* sp. mycelium. However, since this test used a crude allicin extract solution, further testing using pure allicin is needed to confirm this assumption. This experiment also showed that the dosage of crude allicin extract solution influenced the response of *Ganoderma* sp. mycelium. The results indicated that a concentration of 1.4% EC of the crude allicin extract solution inhibited the mycelial growth of *Ganoderma* sp. isolates from both Rejosari and Bekri. These findings are consistent with Sarfraz et al. (2020), who reported that allicin is capable of controlling

pathogens. Observations also showed that it took 19 days (approximately 3 weeks) for the organic fungicide to inhibit the growth of *Ganoderma* sp. fruiting bodies. Further testing with the second stage of organic fungicide application showed continued effects—the mycelial growth remained suppressed, and the fruiting body texture became hard and compact. Similar results were observed in the thickening of mycelium. The development of fruiting bodies treated with *Trichoderma* sp. also showed changes in color, although such changes in color and texture had already occurred after the second stage of organic fungicide application. Research by Elkhateeb and Daba (2022) demonstrated that *Trichoderma Ganodermatigerum* can infect *Ganoderma* sp., thereby inhibiting both its growth and sporulation. In the test

observing microbial growth from the underside of the fruiting body, no bacterial growth was detected. This result indicates that the tested organic fungicide was also capable of killing or inhibiting bacterial growth that coexisted with *Ganoderma sp.* in the lower (lamella) part of the fruiting body.

The viability test comparing fruiting bodies treated with organic and biological fungicides showed that *Ganoderma sp.* could not be isolated from the treated fruiting bodies, while it could be isolated from the control. This confirms that the fruiting bodies treated with the organic fungicide and *Trichoderma sp.* biofungicide had died, or that the combination of organic and biological fungicides successfully killed the *Ganoderma sp.* fruiting bodies. Previous studies by Sarfraz et al. (2020) and Yen and Ali (2022) also showed that both allicin and *Trichoderma sp.* can inhibit or kill *Ganoderma sp.*

Observations conducted 26 days after the breaking of the fruiting bodies revealed that at the treated *Ganoderma sp.* sites, no new fruiting body growth occurred, whereas at the control sites, new fruiting bodies reappeared. This result indicates that viable *Ganoderma sp.* tissues still existed within the wood of the breadfruit tree and that *Ganoderma sp.* had infected the wood deeply into its internal tissue. For comparison, in *Coprinopsis cinerea* (*C. cinerea*), the formation of natural fruiting bodies from the hyphal knot stage to autolysis takes about 48 days (Muraguchi et al. 2015).

Observation results also showed that the growth rate of *Ganoderma sp.* fruiting bodies varied. Under unfavorable conditions, growth slowed down, while under optimal conditions, growth accelerated. In fact, under unfavorable conditions, fruiting body growth could be inhibited, as observed during the incubation period between days 79 and 87. Pilz and Molina (2002) stated that fruiting body growth is influenced by light, humidity, and ambient temperature. Warm temperatures stimulate fruiting body

development more than cool temperatures. Research by Bijalwan et al. (2020) reported that for *G. lucidum* cultivated on poplar wood in Sherpur Village, Himalaya, India, *G. lucidum* fruiting bodies were harvested at 64–66 days, 100–101 days, and 135–136 days for the first, second, and third harvests after log installation, respectively. In another village, Manjgaun, fruiting bodies were harvested at 69–71 days, 107–108 days, and 144–145 days for the respective harvests. The temperature in Sherpur Village was higher than that in Manjgaun Village.

## CONCLUSIONS

The allicin-based organic fungicide effectively inhibited *Ganoderma* mycelial growth in vitro at concentrations above 2% EC and suppressed fruiting body development in vivo within three weeks of application. Combined treatment with *Trichoderma* biofungicide enhanced these effects, resulting in complete mortality of *Ganoderma* fruiting bodies after 3.5 months. The treated tissues turned brown, compact, and non-viable. This eco-friendly approach shows potential for integrated management of basal stem rot in oil palm, pending validation on living plants.

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