

A FIELD EVALUATION ON FUNGICIDES APPLICATION TO CONTROL UPPER STEM ROT (USR) DISEASE IN OIL PALM CAUSED BY *Ganoderma* spp.

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ABSTRACT

Ganoderma spp. causes basal stem rot (BSR) and upper stem rot (USR) diseases in oil palm. In recent years, there has been an increasing report of USR disease in oil palm, which prompted investigation on fungicides application. This study aimed to evaluate the ability of fungicides to control USR-infected palms. The study was conducted on standing oil palm, selected based on the disease severity index (DSI) in the categories of DSI 1 (early infection) and DSI 2 (moderate infection). Two trials were conducted at two different locations in Sarawak (Miri and Sessang), Malaysia. The fungicides, hexaconazole (4.5 g a.i - 90 mL) and tetraconazole (7.5 g a.i. - 60 mL) were dissolved in 3 L of water; applied using a trunk injector three times at six months interval and followed up with disease assessments conducted at six-monthly intervals. The investigation found that more palms succumbed to USR infection in the control treatment than in the treated USR-infected palms. After 60-months post-treatment, the USR-infected palms treated with hexaconazole gave a lower percentage of dead palms at 33.33% (Miri) and 50.00% (Sessang) compared with USR-infected palms treated with tetraconazole at 66.70% (Miri) and 100.00% (Sessang) and untreated USR-infected palms at 100.00%. The hexaconazole-treated USR-infected palms continued surviving and actively producing fruit bunches. In conclusion, hexaconazole was found to be more effective than tetraconazole. It gave a higher survival rate by ceasing the progress of disease development, resulting in lengthening the productive lifespan of USR-infected palms.

Keywords: fungicide, *Ganoderma boninense*, hexaconazole, tetraconazole, upper stem rot.

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INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is an important perennial crop in Malaysia and plays an essential role in the agricultural and economic development of the country (Kushairi *et al.*, 2019; Parveez *et al.*, 2021). Palm oil production in Malaysia has increased over the years, from 4.1 million tonnes in 1985 to

6.1 million tonnes in 1990, and up to 19.86 million tonnes in 2019 (Parveez *et al.*, 2020). However, one of the main constraints faced by the oil palm industry is diseases caused by plant pathogens. Basal stem rot (BSR) is the biggest threat to oil palm production and has been documented to cause huge damage to the oil palm industry in Malaysia. Although BSR is the most prominent disease affecting the oil palm industry, the upper stem rot (USR) disease in oil palm in recent years has seen a sharp rise in incidences, reported mostly in Sabah, Malaysia (Gassner *et al.*, 2005), Sarawak, Malaysia (Rakib *et al.*, 2017) and Indonesia (Hari *et al.*, 2011). A number of *Ganoderma* spp., including *Ganoderma boninense* are known to cause BSR and USR. In USR-infected palm, the infection of *Ganoderma* was found to be on

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Figure 1. Upper stem rot (USR) symptoms; formation of the fruiting body and stem rotting appear at one meter from ground level.

the upper portion of the stem or trunk, appearing at 1 m above ground (Figure 1) as opposed to the BSR symptoms which appear at the base of an infected palm (Rakib *et al.*, 2014).

USR disease is generally accepted to be less common than BSR disease, and its occurrence is very estate-specific (Gassner *et al.*, 2005). Research has proven that BSR disease spreads through root-to-root contact and from one palm to the adjacent palm. It was suggested that USR disease spread is associated with the airborne-basidiospores or insect-transmitted, although the disease initiation remains unknown (Hushiarian *et al.*, 2013). The role of *Ganoderma* basidiospores in disease infection remains unclear. Despite the fact that large numbers of basidiospores are released from basidiomata in oil palm fields, most oil palms are not infected, indicating that the basidiospores are less effective to cause infection, possibly requiring more specific conditions to succeed (Ho and Nawawi, 1986). However, a study conducted by Pilotti *et al.* (2018) revealed that multiple isolates of *G. boninense* were found in seedlings growing near infected palms in the oil palm field, indicating that colonisation of roots by basidiospores and dikaryons, resulting from mating between basidiospores occurred in oil palm plantations.

There are several methods to control *Ganoderma* in BSR, such as cultural practices, including deboling and sanitation, soil mounding and the application of biological control agents (Idris *et al.*, 2016). As a short-term control measure, fungicide application as a curative treatment remains crucial for managing the disease in the fields. Various fungicides have been investigated to control this disease, and systemic fungicides such as drazoxolone, cycloheximide, triadimefon, carbonix, benomyl, hexaconazole, penconazol and tetraconazole were found to be effective based on *in vitro* studies (Arifurrahman and Idris, 2008). Hexaconazole and tetraconazole are classified under the triazole group, a broad-spectrum systemic fungicide. These triazole fungicides were previously reported as a foliar treatment to control apple scab caused by *Venturia inaequalis* (Shahinasi *et al.*, 2017) and powdery mildew in wheat caused by

Blumeria graminis f. sp. *tritici* (Basandrai *et al.*, 2013). These pathogens are fungi and were successfully controlled by fungicide applications. Yaduman *et al.* (2018) observed that a combination of fungicides, carbendazim and propiconazol as a foliar spray and seed treatment was most effective for sheath blight of rice caused by *Rhizoctonia solani*, a soil-borne plant pathogenic basidiomycete. More recently, a new triazole fungicide, mefentrifluconazole, is available for the disease management of *Septoria tritici* blotch disease in wheat, with promising results obtained via *in vitro* assessment (Klink *et al.*, 2021).

A study by Idris *et al.* (2009) assessed the effectiveness of these triazole groups, namely hexaconazole and tetraconazole, in prolonging the productive life of BSR-infected palms. Their findings showed promising results where these fungicides could halt disease progression in infected palms, hence, prolonging the productive life of the palms. It was reported that 74.4% of the palms treated with hexaconazole remained productive by continuously producing fruit bunches for more than five years, while none of the untreated palms survived (Idris *et al.*, 2010). The increased number of USR diseases in existing oil palm requires immediate short-term control measures. Given the success of the fungicides in controlling BSR disease, this study aimed to assess the potential of hexaconazole and tetraconazole in controlling USR-infected palms through trunk injection.

MATERIALS AND METHODS

Study Sites

The study was conducted at two oil palm plantations in Miri, Sarawak (Site 1) and Sessang, Sarawak (Site 2). The palms, 12 years old (first generation) *Dura* x *Pisifera* (DxP) were planted on peat soil in Miri, Sarawak, covering approximately 32.0 ha. Meanwhile, in Sessang, Sarawak, the palms (DxP) selected for the study were 11 years old (first generation), planted on peat soil covering approximately 40.0 ha.

Treatments

Thirty-six USR-infected palms that were still producing fruit bunches in categories DSI 1 and DSI 2 (Table 1) were selected, and the trial was laid down in a completely randomised design (CRD). Twelve healthy oil palms were also included in the study as the negative control. Four treatments were applied, whereby each treatment consisted of 12 oil palms (Table 2). Both fungicides, hexaconazole (4.5 g a.i. - 90 mL) and tetraconazole (7.5 g a.i. - 60 mL) were dissolved in 3 L of water and applied three times at six-month intervals (Idris *et al.*, 2010).

TABLE 1. DISEASE SEVERITY INDEX (DSI) CORRESPONDING WITH LEVELS OF SEVERITY IN MATURE PALM

Disease class	Signs and symptoms of infection
0	Healthy palm. Absence of fruiting body, foliar symptom or stem rotting at the base. Early detection tests (e.g., GSM or PCR-DNA) are negative for the presence of <i>Ganoderma</i> .
1	Early infection or mildly infected palm. Presence of white mycelium or fruiting body (e.g., small white button) or no foliar symptoms with mild or no stem rotting (<10%) at trunk/stem. Early detection methods (e.g., GSM or PCR-DNA) show positive <i>Ganoderma</i> detection.
2	Moderately infected palm. Presence of white mycelium or fruiting body (e.g., small white button or bracket shape). Palm showing foliar symptoms: yellowing and collapsed fronds (<50%) and stem rotting (<30%) at the trunk. Confirmed presence of <i>Ganoderma</i> fungus using early detection methods (e.g., GSM or PCR-DNA).
3	Severely infected palm. Presence of white mycelium or fruiting body (e.g., small white button or bracket shaped form). Palm showing severe foliar symptoms (>50%) and stem rotting (>30%) at the trunk. Confirmed presence of <i>Ganoderma</i> fungus using early detection methods (e.g., GSM or PCR-DNA).
4	Dead palm. Presence of white mycelium or fruiting body (e.g., small white button or bracket shaped form). Collapsed/dead palm with severe foliar symptoms and stem rotting at the trunk. Confirmed presence of <i>Ganoderma</i> fungus using early detection methods (e.g., GSM or PCR-DNA).

Note: GSM - *Ganoderma* selective medium; PCR-DNA - polymerase chain reaction-deoxyribonucleic acid.

Source: Idris *et al.* (2016).

TABLE 2. TREATMENTS OF FUNGICIDES APPLIED TO UPPER STEM ROT (USR) - INFECTED PALMS

Treatments	Description	No. of palms
T1	USR-infected palm treated with hexaconazole - 4.5 g a.i. (90 mL) + 3 L water	12
T2	USR-infected palm treated with tetraconazole - 7.5 g a.i. (60 mL) + 3 L water	12
T3	Positive Control – Untreated USR-infected palm	12
T4	Negative Control – Untreated healthy palm	12

Trunk injection was carried out using a hand knock injector attached to the motorised knapsack sprayers to pressure-inject the fungicide solution into a hole made on the trunk (2 L/hole) at one-meter height from the ground level. The remaining 1 L of the fungicide solution was sprayed directly onto the USR-infected trunk (Figure 2).

Disease Assessments

The presence of *Ganoderma* in the trunk tissue was confirmed by culturing the collected sample on *Ganoderma* selective medium (GSM), prepared according to Idris *et al.* (2016). The palms status in each of the treatments was assessed at six-monthly intervals over five years by recording the presence or absence of USR symptoms, including external symptoms (e.g., foliar symptoms and appearance of *Ganoderma* fructification) and decay of the trunk. Oil palms were scored according to disease class, ranging from 0-4 (Table 3). Disease severity of foliar index (DSFI) was calculated based on the symptoms appearing on the foliage (external), according to Liu *et al.* (1995) [Equation (1)].

$$DSFI (\%) = \frac{\sum (\text{Number of palms in the scale} \times \text{Severity rating})}{\text{Total number of palms assessed} \times \text{Highest rating}} \times 100 \quad (1)$$

The extent of disease developed over time for each treatment was further expressed as the area under the disease progress curve (AUDPC), calculated based on a Equation (2) as described by Campbell and Madden (1990):

$$AUDPC = \sum_i^{n-1} [(y_i + y_{i+1})/2] (t_{i+1} + t_i) \quad (2)$$

where n is the number of assessments, y is the measurement of disease (DSFI) and t is the time of observation. Disease reduction (DR) was calculated based on the value of AUDPC by the following Equation (3):

$$DR (\%) = \frac{(\text{AUDPC control} - \text{AUDPC treatment})}{\text{AUDPC control}} \times 100 \quad (3)$$

The assessment was also carried out based on the quantitative assessment measured by inspection of the dead palms. All data in percentage were arcsine transformed (Gomez and Gomez, 1984) and subjected to analysis of variance (ANOVA) with the means compared by LSD at $p \leq 0.05$ using Statistical Analysis System (SAS® Software) (SAS Institute Inc).



Figure 2. Application of fungicides on the upper stem rot (USR)-infected palm (a) selection of USR infected-palm with disease severity index (DSI) 1 and 2 and still producing fruit bunches, (b) removal of the old frond and fruiting bodies from the USR-infected stem/trunk tissues using a chisel, (c) drilling of one hole on the stem/trunk at 1-m height above ground level using motorised engine drill attached with a drill bit (45 cm long, 11 mm diameter), (d) injecting a total of 2 L/palm of fungicide solution using hand knock injector attached to the motorised knapsack sprayers and (e) spraying remaining 1-L fungicide solution onto the USR stem/trunk infected tissues.

TABLE 3. SIGNS AND SYMPTOMS OF DISEASE SEVERITY FOLIAR INDEX (DSFI) SCORED ON A SCALE OF 0-4

Disease class	Signs and symptoms of infection
0	Healthy palms with green leaves and without the appearance of fungal mycelium on any part of the plant.
1	Presence of white mycelium or fruiting body on any part of the plant without necrosis or chlorosis of leaves.
2	Presence of white mycelium or fruiting body on any part of the plant with necrosis or chlorosis of leaves (<25%).
3	Presence of white mycelium or fruiting body on any part of the plant with necrosis or chlorosis of leaves (<75%).
4	Presence of white mycelium or fruiting body on any part of the plant with necrosis or chlorosis of leaves (>75%) or the palm is dead.

Source: Izzati and Abdullah (2008).

RESULTS AND DISCUSSION

The current field study was conducted between 2014 and 2020 to evaluate the response of *Ganoderma*-USR infected palms upon the application of hexaconazole and tetraconazole. Isolates of *Ganoderma* were isolated from the USR infected trunk tissues on GSM (Figure 3). The formation of a brown halo on the GSM media confirmed the presence of basidiomycete fungi including *Ganoderma* (Naidu *et al.*, 2018). The data was presented annually for five years, although the census was carried out at six-monthly intervals. The symptoms of USR infection were observed with the appearance of white mycelium, which later developed into the white button and fruiting body along the stem or trunk. Before the application of the fungicides, the DSFI of each palm within each treatment was found to be similar, with no significant differences recorded (Figure 4). Twelve months after application (MAA), all the treated palms in both study sites significantly showed lower DSFI with hexaconazole, T1 (10.8%-17.2%) and tetraconazole, T2 (14.6%-18.9%) compared to the untreated palms in T3 (18.6%-37.7%). In Site 1 (Miri), the difference between the

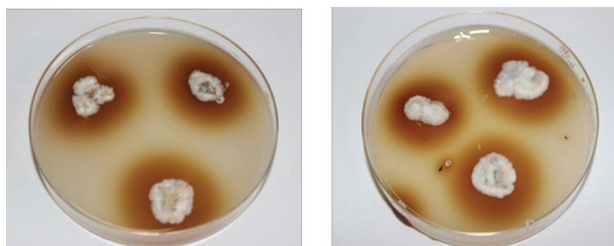
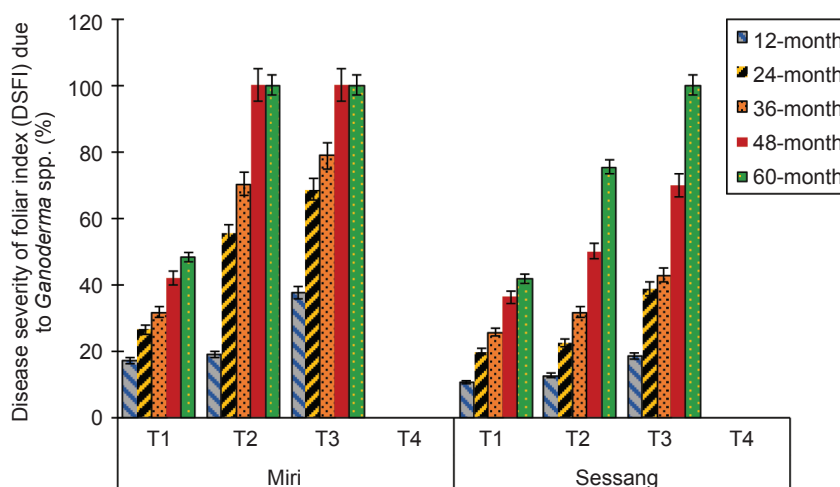


Figure 3. Infected tissues from USR-infected palms cultured on *Ganoderma* Selective Medium (GSM).

DSFI percentage of T1 and T2 became bigger at 48 MAA where it was observed that 100.0% of DSFI was recorded in all palms in T2. On the contrary, palms of T2 in Site 2 (Sessang), showed a significantly lower infection symptom than the untreated palms (T3). It was observed that after 48 months, untreated palms (T3) showed DSFI significantly higher at 70.0% and 100.0% for both study sites, as compared to DSFI of only 36.3%-42.1% in the palms treated with hexaconazole, T1. At 60 MAA, the palms in T1 showed the lowest DSFI percentage ranging from 41.7%-48.1%, highlighting the potentials of hexaconazole in suppressing the disease. Meanwhile, no fruiting bodies, white buttons or mycelia were observed on the control palms in T4 (healthy).

DSFI data was further evaluated by AUDPC (Table 4). The AUDPC values suggest the amount of disease development in each treatment whereas the treatment with the lowest AUDPC value indicate the effectiveness of the treatment in reducing the disease. The treated palms in T1 exhibited significantly lowest AUDPC of 132.96 unit² and 108.02 unit² in Site 1 and Site 2, respectively. This was followed by T2 with AUDPC values of 284.98 unit² and 148.35 unit², respectively. It was then followed by T3 with significantly the highest AUDPC values of 316.35 unit² and 210.80 unit². The highest DR for both sites were indicated by T1 treatment with 48.76% and 57.97% reduction compared to the control.

The disease assessment data in Figure 5 represents the percentage of dead palms due to *Ganoderma* infection observed until 60 MAA. In both study sites, there were no dead palms recorded on palms treated with hexaconazole and tetraconazole at the beginning. Twelve months post-application, it was recorded that the percentage of dead palms was



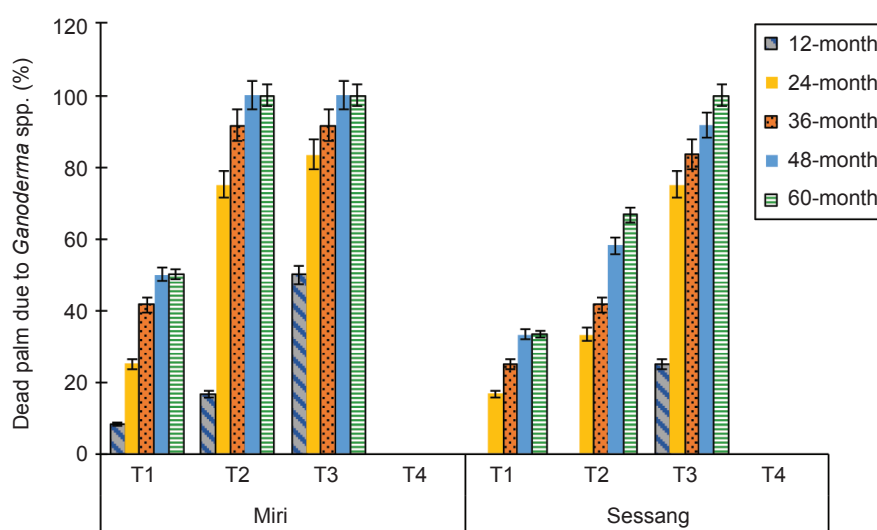
Note: Values are represented by the mean (n=12), with vertical bars representing standard error. T1 is the USR-infected palms treated with hexaconazole at 4.5 g a.i. (90 mL) + 3 L water; T2 is the USR- infected palms treated with tetraconazole at 7.5 g a.i. (60 mL) + 3 L water; T3 is the control (untreated USR-infected palms); T4 is the control (untreated healthy palms).

Figure 4. Percentage of disease severity of foliar index (DSFI) due to upper stem rot (USR) disease at the study sites, Miri and Sessang.

TABLE 4. AREA UNDER THE DISEASE PROGRESS CURVE (AUDPC) AND DISEASE REDUCTION (DR) DUE TO UPPER STEM ROT (USR) DISEASE IN OIL PALM AFTER FUNGICIDE TREATMENT

Treatments	Site 1 (Miri)		Site 2 (Sessang)	
	The area under the disease progress curve (AUDPC) (unit ²)	Disease reduction (DR) (%)	The area under the disease progress curve (AUDPC) (unit ²)	Disease reduction (DR) (%)
T1	132.96 ± 30.50a	57.97	108.02 ± 12.77a	48.76
T2	284.98 ± 41.20b	9.92	148.35 ± 21.10b	29.63
T3	316.35 ± 22.50c		210.8 ± 32.70c	
T4	0	-	0	-

Note: Means followed by the same letter within a column are significantly not different ($p \leq 0.05$) ($n=12$). T1 is the USR-infected palms treated with hexaconazole at 4.5 g a.i. (90 mL) + 3 L water; T2 is the USR- infected palms treated with tetraconazole at 7.5 g a.i. (60 mL) + 3 L water; T3 is the control (untreated USR-infected palms); T4 is the control (untreated healthy palms).



Note: Values are represented by the mean ($n=12$), with vertical bars representing standard error. T1 is the USR-infected palms treated with hexaconazole at 4.5 g a.i. (90 mL) + 3 L water; T2 is the USR- infected palms treated with tetraconazole at 7.5 g a.i. (60 mL) + 3 L water; T3 is the control (untreated USR-infected palms); T4 is the control (untreated healthy palms).

Figure 5. Percentage of dead palms due to upper stem rot (USR) disease at the study sites, Miri and Sessang.

lower in both treated palms of T1 (hexaconazole) and T2 (tetraconazole) in Site 1 (Miri) while there was no dead palm recorded in Site 2 (Sessang). Interestingly, the dead palms recorded in T2 were three times higher compared to dead palms in T1 after 24 MAA at Site 1, and this value continued to increase to 100.0% after 48 MAA. A similar situation was also observed in Site 2, as the percentage of dead palms in T2 was significantly higher compared to T1. On the other hand, untreated palms (T3) showed significantly the highest percentage of dead palms over the years. Based on this study, the percentage of dead palms was significantly lowest at $p < 0.05$ in palms treated with hexaconazole (T1) (33.3%-50.0%) after 60 MAA.

This study presents the first report on USR disease control in oil palm through fungicides application. One common characteristic between USR-infected and BSR-infected palms is that both

diseases are caused by a similar pathogen, *Ganoderma* spp. The pathogen can cause a significant increase of incidences in the affected area. The difference between USR and BSR is that the former infects the palm approximately 1 m above the stem base, while the latter occurs at the stem base of the oil palm (Rakib *et al.*, 2014). Generally, in the initial stage of USR infected palm, the yellowing of leaves will be accompanied by the formation of sporophores at the oil palm trunk. In the final stage of infection, severe desiccation of leaves and the production of fruiting bodies at the mid-section of the trunk will be observed.

The field trial was conducted on USR-infected palms with early symptoms of infection with continuous production of fruit bunches. Visual-based disease observation is still the only method to identify the USR disease in the field (Idris, 2011; Rakib *et al.*, 2015). Most fungicides need to be applied before

the onset of disease or at the first appearance of the disease symptoms for effective control (Poole and Arnaudin, 2014). For instance, research on managing northern corn leaf blight (NCLB) suggested that the appropriate time for trifloxystrobin and epoxiconazole fungicides application in reducing NCLB was at the onset of disease (severity below 2%) (Carpane *et al.*, 2020). In the current study, the use of fungicides was shown to prolong the economic lifespan of the diseased palms as it suppressed the spread of *Ganoderma* infection within the infected area of standing palms in category of DSI 1 and DSI 2. Although *Ganoderma* infection cannot be eliminated, the economic life of infected palms was prolonged with continuous production of fresh fruit bunches (FFB) due to the effective chemical control. Having said this, another setback in most fungicides' is the fact that the effect is temporary. More applications and higher dosages are likely to improve the disease control; however, the number of applications and dosage must be optimised to achieve efficient disease control with a prolonged effective lifespan of the plant (Van den Berg *et al.*, 2016). This study suggested that repeated application of the fungicide is necessary in the event of a relapse of infection within the oil palms. Therefore, infection of adjacent healthy tissues by the fungus would be halted with repeated applications of systemic fungicide over a period of time. This method was successfully demonstrated by Thangeswari *et al.* (2019), who found that repeated fungicides application was effective in the management of BSR disease in coconut. The application of hexaconazole (5.0% EC) and tetraconazole (3.8% EC) as root feeding at a quarterly interval in BSR-infected coconut palm recorded lower disease incidence at 3.28% and 4.86% when compared to 17.40% in control.

Fungicides application contributes significant importance to crop protection (Oliver and Hewitt, 2014). Hexaconazole, a systemic fungicide, is used to control a broad spectrum of diseases and is more productive and cost-effective than other triazole fungicides. The fungicide, which is a potent inhibitor of ergosterol biosynthesis (Huang *et al.*, 2012), is active mainly against basidiomycetes and ascomycetes. The triazole fungicides can inhibit a specific enzyme, C14-demethylase, which plays an important role in sterol production (Yan *et al.*, 2016). Sterols, such as ergosterol, are required for membrane structure and function, making them essential for developing functional cell walls in fungi. The sterols are extremely crucial that their deficiency results in abnormal fungal growth and eventually death in fungi (Alcazar-Fuoli and Mellado, 2013). In the present work, disease manifestation through DSFI was lower in USR-infected palms treated with hexaconazole (T1) compared to the percentage recorded in USR-infected palms treated with tetraconazole (T2) and untreated control (T3). This

finding showed that hexaconazole application prolonged the lifespan of the treated palms and significantly reduced the number of dead palms. Similar findings were reported in rice, whereby Kumar *et al.* (2013) found that the application of hexaconazole at 75% WG@50 g a.i./ha, gave effective control of sheath blight with increased yield when compared to the untreated plot. Recently, a new chitosan nanoparticles formulation containing the active ingredient of hexaconazole was successfully developed and showed promising results with a significant percentage reduction of disease in oil palm seedlings infected with *G. boninense* (Maluin *et al.*, 2020). This observation suggested that *Ganoderma* infection in treated oil palms was slowed down, particularly in palms treated with hexaconazole, hence, delaying the appearance of foliar symptoms.

Previous studies have also indicated that hexaconazole was able to treat BSR disease in oil palms (Idris, 2011; Maluin *et al.*, 2019). Similarly, tetraconazole was reported to reduce the BSR incidence in oil palm (unpublished data) and coconut (Thangeswari *et al.*, 2019). However, the use of tetraconazole in the present study was ineffective against USR-infected palms. This was evident with the application of tetraconazole in Site 1 (Miri) which failed to reduce the disease severity in infected palms giving a higher number of dead palms. The possible reasons for these findings remain unclear, but the failure of tetraconazole to control the disease has been found in this study. Additionally, this result is in line with that of Arabiat (2015), who evaluated tetraconazole for the management of *Aphanomyces cochlioides* in sugarbeet. The study concluded that this fungicide was ineffective at controlling *A. cochlioides* in the *in vivo* assessment. However, it was capable of reducing the disease based on the *in vitro* study. Different fungicide products may have different levels of biological efficacy against the same pathogen. Ramdial *et al.* (2016; 2017) highlighted several reasons that might be associated with fungicide failure and resistance due to multiple interacting factors. Among these were repetitive use and exceeding dose rate, and inaccuracy in the application interval. The application of tetraconazole in USR-infected palms did not improve the level of disease control provided by trunk injection applications. It may require a higher dosage, but this remains to be investigated in the future. In some cases, some products can be effective for the control of a specific disease but differ in their effectiveness. A study on fruit anthracnose disease of the chili plant was conducted using two fungicides, namely tebuconazole and hexaconazole. The study results gave varying control efficacy, with hexaconazole being superior at 83.3% disease control (Anand *et al.*, 2020). This study should be explored further to confirm the consistency of the results and possibly with the mixture application of two different

fungicides or a higher dosage with a varied interval of applications.

CONCLUSION

Hexaconazole can be recommended to control USR disease in oil palms. This is shown by the significant reduction of dead oil palms, and lower DSFI observed after hexaconazole treatment compared to the non-treated control oil palms. The recommended rate is 4.5 g a.i. (90 mL) + 3 L water per palm, combining the trunk injection and spray application at the infected trunk, repeated three times at six-monthly intervals. The application of hexaconazole on USR-infected palms demonstrates that it could control the progress of USR disease development hence, prolonging the life of diseased palms.

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