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IN VIVO TESTING OF *Pseudozyma hubeiensis* TO CONTROL *Aspergillus flavus* IN NUTMEG SEEDS (*Myristica fragrans* Houtt.)

PENGUJIAN *Pseudozyma hubeiensis* SECARA *IN VIVO* UNTUK MENGENDALIKAN *Aspergillus flavus* PADA BIJI PALA (*Myristica fragrans* Houtt.)

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Abstract

Nutmeg (*Myristica fragrans* Houtt.) is a high-value spice commodity from Indonesia, often contamination with aflatoxin-producing *Aspergillus flavus*. Previous research has identified two epiphytic yeast isolates from nutmeg plants, namely DP1341a and DP1342, both of which were identified as *Pseudozyma hubeiensis* and showed antagonistic potential against *A. flavus*. This study is a follow- up study that aims to test the antagonistic activity of both isolates against *A. flavus in vivo* on nutmeg seeds. The research was conducted experimentally through several stages, namely testing the compatibility of isolates on Yeast Malt Agar (YMA) media, determining the optimum fermentation time, and applying liquid culture and fermentation filtrate to 120 nutmeg seeds. Results showed that the consortium of isolates DP1341a and DP1342 did not increase the inhibitory power results compared to single cultures with optimum fermentation times of 10 and 14 days, respectively. *In vivo* applications show that treatment with DP1342 isolate (KC-DP1342) *and* consortium in liquid culture provides the highest level of resistance to *A. flavus* infection, namely 38.67% and 28.00%. The findings suggest that *P. hubeiensis* DP1342 has potential as a biological control agent in controlling *A. flavus* on nutmeg seeds, although further formulation and optimization are needed.

Keywords: Aflatoxin; *Aspergillus flavus*; Biological control; *Myristica fragrans; Pseudozyma hubeiensis*

Abstrak

Biji pala (Myristica fragrans Houtt.) merupakan komoditas bernilai tinggi dari Indonesia, yang rentan mengalami penurunan kualitas akibat kontaminasi Aspergillus flavus penghasil aflatoksin. Penelitian sebelumnya telah mengidentifikasi dua isolat ragi epifit dari tanaman pala, yakni DP1341a dan DP1342, yang keduanya diidentifikasi sebagai Pseudozyma hubeiensis dan menunjukkan potensi antagonistik terhadap A. flavus. Penelitian ini merupakan studi lanjutan yang bertujuan untuk menguji aktivitas antagonistik kedua isolat tersebut terhadap A. flavus secara in vivo pada biji pala. Penelitian dilakukan secara eksperimental melalui beberapa tahapan, yaitu uji kompatibilitas isolat pada media Yeast Malt Agar (YMA), penentuan waktu fermentasi optimum, serta aplikasi kultur cair dan filtrat fermentasi terhadap 120 biji pala. Hasil menunjukkan bahwa konsorsium isolat DP1341a dan DP1342 tidak meningkatkan hasil daya hambat dibanding kultur tunggal dengan waktu fermentasi optimum masing-masing selama 10 dan 14 hari. Aplikasi in vivo menunjukkan bahwa perlakuan kultur cair isolat DP1342 (KC-DP1342) dan kultur cair konsorsium (KC-Konsorsium) memberikan tingkat ketahanan terhadap infeksi A. flavus tertinggi, yaitu sebesar 38,67% dan 28,00%. Temuan ini menunjukkan potensi penggunaan P. hubeiensis DP1342 sebagai agen hayati dalam pengendalian A. flavus pada biji pala, meskipun formulasi dan optimasi masih diperlukan.

***Kata Kunci**: Aflatoksin; Aspergillus flavus; Biokontrol; Myristica fragrans; Pseudozyma hubeiensis*

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INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt.) is a spice plant native to the Banda and Maluku Islands. Indonesia is the world's leading exporter of nutmeg, supplying approximately 70–75% of global demand (Malda et al., 2022). However, the quality of Indonesian nutmeg products remains relatively low, primarily due to contamination by aflatoxin-producing *Aspergillus flavus*. Between 2016 and 2020, there were 16 notifications from European Union importing countries regarding non-compliance with health certificates and analytical reports for Indonesian spice exports, particularly nutmeg (Owolabi & Olayinka, 2021). These issues were largely attributed to contamination with mycotoxins, especially aflatoxins, as further confirmed by several rejections of nutmeg shipments by EU countries throughout 2021 due to aflatoxin and ochratoxin A contamination (Rapid Alert System for Food and Feed (RASFF) Portal, 2022).

Aflatoxins are toxic secondary metabolites produced primarily by *Aspergillus flavus*. Continuous consumption of aflatoxin-contaminated products can lead to poisoning, liver damage, immune suppression, and even death (Mikasari et al., 2015). Various methods have been employed to control *A. flavus* contamination, including physical treatments such as drying (Sembiring et al., 2020) and gamma-ray irradiation (Nurtjahja et al., 2018). Chemical approaches include fumigation (Kasim et al., 2017), coating with specific formulations (Rahayuningsih et al., 2022; Supriadi et al., 2022), and the use of synthetic fungicides (Zahara et al., 2021). Although synthetic fungicides are often more effective, their use may leave harmful residues that can contaminate food products and negatively impact the environment (Goswami, 2018).

Therefore, the development of environmentally friendly control methods is crucial. One promising alternative is the use of plant-origin, non-pathogenic antagonistic yeasts as biological control agents or biofungicides. These microorganisms have the potential to promote plant growth, enhance plant-environment interactions, and protect against pests and pathogens (Wahyu et al., 2019). Research by Moradi et al. (2020) identified 13 indigenous yeast isolates from soil and pistachio seeds that effectively reduced aflatoxin B1 production by 90.6–98.3%. Similarly, Jaibangyang et al. (2020) discovered 49 epiphytic and endophytic yeast strains capable of inhibiting *A. flavus* growth via volatile organic compound (VOC) production.

Deswanti (2021) conducted a preliminary study on yeast isolated from nutmeg plants for its potential to control *A. flavus*. Five yeast isolates demonstrated inhibitory activity against *A. flavus in vitro*, with the two most effective isolates, DP1341a (47.23%) and DP1342 (55.98%), both identified as *Pseudozyma hubeiensis*. These two isolates were believed to originate from different strains, based on their sequence similarity values of 100% and 99.84% in the NCBI GenBank database.

Despite promising *in vitro* results, the application of these yeast isolates has not yet been evaluated *in vivo* on nutmeg seeds. Therefore, this study aims to assess the antagonistic potential of *Pseudozyma hubeiensis* isolates DP1341a and DP1342 *in vivo*. Treatments involved both liquid cultures and fermented filtrates, applied as single isolates and as a consortium. Prior to consortium application, compatibility testing was conducted to ensure synergistic interaction between isolates. Additionally, determining the optimal fermentation time was essential for maximizing antifungal compound production in the fermented filtrate treatments.

The contamination of nutmeg seeds can begin during the open-air drying process, which uses sunlight. Furhermore, nutmeg seeds are sold open in traditional markets and sealed in plastic in supermarkets. Therefore, this research provides initial insights into the effectiveness of antagonistic yeast isolates in suppressing *A. flavus* infection on nutmeg seeds. By comparing the efficacy of liquid cultures and fermented filtrates, the study aims to identify the most effective treatment approach. The findings are expected to support the development of yeast-based biofungicides as sustainable biological control agents for maintaining the quality of nutmeg commodities.

MATERIALS AND METHODS

The materials used in this study included pure cultures of the yeast *Pseudozyma hubeiensis* (isolates DP1341a and DP1342), originally isolated from the leaf surface of nutmeg (*Myristica fragrans* Houtt.) and obtained from the BB-Biogen Laboratory. A toxigenic strain of *Aspergillus*

flavus (BIO33210) capable of producing aflatoxin was also obtained from BB-Biogen. Aged nutmeg seeds were collected from nutmeg plantations in the North Sulawesi region.

Yeast Isolate Compatibility Test

Compatibility between isolates DP1341a and DP1342 was evaluated using the perpendicular streak method on yeast malt agar (YMA). A loopful of each isolate was streaked perpendicular to each other on YMA plates and incubated at room temperature for 2 days. The presence or absence of an inhibition zone at the intersection of growth was observed. A lack of an inhibition zone and uninterrupted growth indicated potential compatibility and synergism for consortium application (Haque et al., 2021). If an inhibition zone was present, the combination treatment (consortium) was excluded from further testing.

Determination of Optimum Fermentation Time for Antagonistic Activity

To determine the optimal fermentation time for maximum antifungal compound production, a loopful of each isolate (DP1341a and DP1342) was inoculated into 10 mL of yeast malt broth (YMB) in 50 mL Erlenmeyer flasks and incubated at 27 °C with shaking at 120 rpm for 2, 4, 6, 8, 10, 12, and 14 days (Mhetras et al., 2019). A 0.1 mL suspension of *A. flavus* conidia (1.0×10^6 conidia/mL) was added to 10 mL sterile PDA media (40 °C) and poured into the sterile petri dish (9 mm). From each fermentation time point, 1 mL of culture was centrifuged at 8,000 rpm for 10 minutes. The supernatant was pasteurized at 60 °C for ±25 seconds, and 20 µL of the pasteurized filtrate was applied to 8 mm sterile paper discs. The discs were placed on the PDA quadrants alongside positive (benomyl) and negative (sterile water) controls. The treatment was repeated twice. Cultures were incubated at room temperature for 4 days, and the diameter of the inhibition zones was measured. The fermentation time point showing the largest inhibition zone was considered optimal for each isolate (Rusli et al., 2017). The diameter of the inhibition zone is divided into very strong (inhibition zone more than 20 mm), strong (inhibition zone 10–20 mm), moderate (inhibition zone 5–10 mm), and weak (inhibition zone less than 5 mm) Davis and Stout (1971), *in* Safitri et al. (2017).

Preparation of Yeast Liquid Cultures and Consortium

For application, each isolate (DP1341a, DP1342) and its consortium (DP1341a + DP1342) were cultivated by inoculating two loopfuls into 100 mL YMB in 250 mL Erlenmeyer flasks. Cultures were incubated at 27 °C with shaking at 120 rpm for 2 days. These cultures were directly used for application as a liquid inoculum.

Fermentation and Filtrate Preparation

To produce fermented filtrates, each isolate was grown separately in 120 mL of YMB by inoculating two loopfuls of culture. Fermentation was carried out in an orbital shaker at 27 °C, 120 rpm, for 10 days (DP1341a) and 14 days (DP1342), based on previous optimum time findings. The resulting cultures were centrifuged at 4,000 rpm for 15 minutes. The supernatant was collected and pasteurized in a water bath at 60 °C for ±25 minutes to eliminate residual live cells without degrading antifungal metabolites (Harni et al., 2016). The pasteurized supernatant was stored in sterile containers for use in further assays.

Application to Nutmeg Seeds

A total of 120 nutmeg seeds with intact shells were washed under running water, surface sterilized with 70% ethanol for 1 minute, soaked in 3% sodium hypochlorite (NaOCl) for 3 minutes, and rinsed three times with sterile distilled water (Hanif & Susanti, 2017). Fifteen seeds per treatment group were soaked in 100 mL of either yeast liquid culture, fermented filtrate, sterile YMB (negative control), or 0.1% benomyl (positive control) for 24 hours. Seeds were then air-dried in a laminar airflow chamber for approximately 3 minutes.

Subsequently, seeds were inoculated using a mini-sprayer with *A. flavus* conidia suspension (1.0×10^6 conidia/mL), spraying approximately 0.5 mL (10–11 sprays) per 15 seeds. The treated seeds were incubated at room temperature in sterile jars for 7 days. After incubation, visual

observation of *A. flavus* colony growth on the seed surface was conducted, and colonization rates were assessed (Vincelli & Hershman, 2011). The treatment was repeated two times, and each plate consisted of five of nutmeg seeds.

Assessment of Infection Incidence, Severity, and Inhibition

After 7 days, infection symptoms were visually evaluated based on the appearance of greenish-yellow to dark green mold on the seed surface (Supriadi et al., 2022). The incidence of infection (KI) was calculated (Table 1) using the formula $\mathrm{KI} = \frac{\mathrm{n}}{\mathrm{N}}$ x 100%. Where KI= infection incidence, n= number of infected seeds; and N= The total number of seeds observed. The severity of infection (KP) was assessed using a numerical scale adapted from Vincelli and Hershman (2011) and calculated using the formula (Gustina et al., 2016), $\mathrm{KP} = \frac{\sum V_i \times n_i}{\mathrm{z} \times \mathrm{N}}$ x 100%. Where Vi= severity score; ni= number of seeds in each severity category; z= maximum severity score; and N= total number of seeds.

Table 1. Severity scoring of nutmeg seed infection by *Aspergillus flavus*

Infection percentage (%)	Score
0	0
>0–20	1
>20–40	2
>40–60	3
>60–80	4
>80–100	5

Under UV light, infected seed surfaces exhibit a yellowish or fluorescent blue glow indicating *A. flavus* or aflatoxin presence. The degree of infection inhibition (DHI) (Table 2) was calculated as $\mathrm{DHI} = \mathrm{D1} - \mathrm{D2}$. Where DHI= degree of infection inhibition; D1= infection severity in negative control; and D2= infection severity in treatment group.

Table 2. Effectiveness category of yeast antagonism against *Aspergillus flavus* (Elfina et al., 2017)

Inhibition (%)	Interpretation
0	Ineffective
0–20	Very poor
20–40	Poor
40–60	Moderate
60–80	Effective
>80	Highly effective

Data Analysis

Data on infection incidence, severity, and inhibition were analyzed using one-way analysis of variance (ANOVA) at a 95% confidence level using SPSS version 26. Significant differences between treatments were further analyzed using Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$.

RESULTS

Compatibility Test Between Yeast Isolates

The compatibility test was performed between isolates DP1341a and DP1342. Figure 1 showed no visible inhibition zone at the intersection point on YMA medium, indicating the absence of antagonism between the two isolates.

Optimal Fermentation Time of Yeast Isolates

The antifungal activity of fermented inoculum from DP1341a and DP1342 showed that the widest average inhibition zones occurred on days 10 and 14 of fermentation, respectively. DP1341a reached a diameter of 12.5 mm (strong category), while DP1342 reached 6.3 mm (moderate category). The positive control (Benomyl) showed an average inhibition zone of 31.6 mm, while the negative control (sterile water) showed no inhibition (Figure 2; Table 3).

***In Vivo* Application of Liquid Culture and Fermented Filtrate**

Yeast isolates were applied to nutmeg seeds using both liquid culture and fermented filtrate methods. All treatments showed 100% infection incidence. However, significant differences in infection severity and inhibition were observed (Table 4). Based on the ANOVA output above, the sig. value is 0.00 <0.05, the average infection inhibition power, infection severity, and infection incidence of nutmeg seeds is different, then continued with the Duncan test. The results of the Duncan test showed that only the positive control treatment (Benomyl) had an average incidence of infection in nutmeg seeds that was significantly different. The results of the Duncan test that the average percentage of infection inhibition and severity in nutmeg seeds between each treatment was significantly different and the two best treatments were KC-DP1342 and KC-consortium based on the Duncan result.

Figure 1. Compatibility test between yeast isolate DP1341a (horizontal) and DP1342 (vertical). Note: the circle indicates the intersection point

Table 3. Inhibition zone diameters (mm) of yeast isolates against *Aspergillus flavus* conidia (1.0 × 10^6 conidia/mL)

Inoculum age (day)	Average of inhibition zone diameter (mm)			
	Control (+)	Control (-)	DP1341a	DP1342
2	31.60 ± 3.74	0.00	0.00	0.00
4	31.60 ± 3.74	0.00	9.00 ± 15.06	0.00
6	31.60 ± 3.74	0.00	2.70 ± 6.53	0.00
8	31.60 ± 3.74	0.00	0.00	0.00
10	31.60 ± 3.74	0.00	12.50 ± 10.80	1.30 ± 3.26
12	31.60 ± 3.74	0.00	0.00	0.00
14	31.60 ± 3.74	0.00	1.30 ± 3.26	6.30 ± 7.40

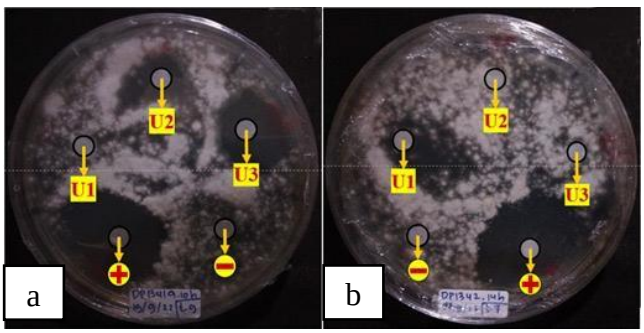

Figure 2. Inhibition zones in Potato Dextrose Agar (PDA) medium inoculated with *Aspergillus flavus*, DP1341a (10-day fermentation) (a) and DP1342 (14-day fermentation) (b). U1= repeat 1; U2= repeat 2; and U3= repeat 3

Further statistical testing using Duncan's test shows the highest value, which has a significant difference with the highest average value. Based on the results of Duncan's test on the average percentage of infection inhibition power in nutmeg seeds between each treatment was significantly different, it was found that KC-DP1342 and KC-Consortium shows the largest diameter colony and is statistically significantly different compared to other isolate. Liquid culture treatments showed greater inhibition of *A. flavus* than fermented filtrates (Figures 3 & 4). DP1342 (KC-DP1342) showed

the highest inhibition (38.67%), followed by the consortium (28.00%) and DP1341a (21.33%). Fermented filtrate treatments showed lower inhibition, ranging from 16.00% to 18.67% (Table 4).

Table 4. Average infection incidence, severity, and inhibition of *Aspergillus flavus* on nutmeg seeds

Treatment	Infection incidence (%)	Infection severity (%)	Inhibition (%)	Effectiveness
Benomyl (+)	13.33 ± 11.54^{a}	2.67 ± 2.31^{a}	88.00 ± 8.00^{c}	+++++
YMB sterile (-)	100.00 ± 0.00^{b}	90.67 ± 6.11^{d}	0.00 ± 0.00^{a}	-
KC-DP1341a	100.00 ± 0.00^{b}	69.33 ± 9.24^{c}	21.33 ± 15.14^{ab}	++
KC-DP1342	100.00 ± 0.00^{b}	52.00 ± 8.00^{b}	38.67 ± 8.32^{b}	++
KC-consortium	100.00 ± 0.00^{b}	62.67 ± 19.73^{bc}	28.00 ± 24.33^{b}	++
FHF-DP1341a	100.00 ± 0.00^{b}	72.00 ± 0.00^{c}	18.67 ± 6.11^{ab}	+
FHF-DP1342	100.00 ± 0.00^{b}	72.00 ± 4.00^{c}	18.67 ± 6.11^{ab}	+
Combined Filtrate	100.00 ± 0.00^{b}	74.67 ± 6.11^{c}	16.00 ± 12.00^{ab}	+

Note: Values with different letters indicate significant differences based on DMRT at α= 0.05. KC= liquid culture; FHF= fermented filtrate

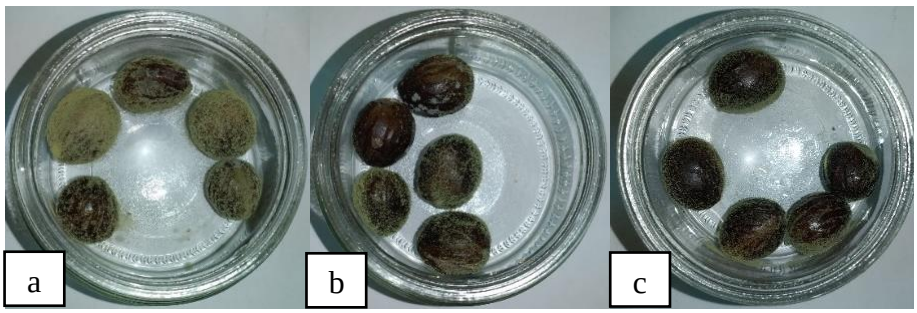

Figure 3. *In vivo* application of liquid culture DP1341a (a), DP1342 (b), and consortium (c)

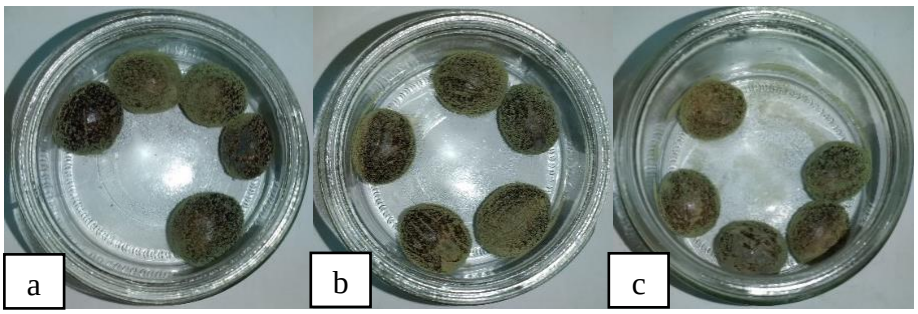

Figure 4. *In vivo* application of fermented filtrate DP1341a (a), DP1342 (b), and consortium (c)

DISCUSSION

The compatibility test showed no inhibition zone between DP1341a and DP1342 isolates, suggesting the absence of antagonistic interaction. This indicates that the two isolates can potentially be co-cultured without inhibiting each other's growth. However, further investigation is required to determine whether the interaction is synergistic or neutral, as co-inoculation does not always guarantee enhanced efficacy (Xu et al., 2011). Table 3 and Figure 2 show that the largest average inhibition zones were obtained in the inoculum of isolates DP1341a and DP1342 on the 10^{th} and 14^{th} days after inoculation, with average diameters of 12.5 and 6.3 mm, respectively. The average diameter results for both isolates were included in the strong (12.5 mm) and moderate (6.3 mm) categories. The formation of inhibition zones on day 10 and 14 after inoculation is thought to be due to both isolates being in the stationary phase; in this phase, the culture has produced secondary metabolites as antifungals in optimum amounts. According to Tanimura et al. (2016) found that *P. hubeiensis* isolates were often found in the stationary phase on day 10 after inoculation on mixed-sugar medium, producing the largest clear zone. Rusli et al. (2017) stated that the widest inhibition zone value indicates maximum antifungal compound production at the optimum fermentation time.

Table 4 also shows that all liquid culture and fermentation filtrate treatments had a positive effect in inhibiting the growth of *A. flavus* on nutmeg seeds. Lower infection severity values in the test treatments indicated higher and better infection inhibition capabilities. This can be seen in the

KC-DP1342 treatment, which provided the best results, with the second-lowest infection severity percentage after the positive control at 52.00% and the highest inhibition percentage among the test treatments at 38.67%. This is in line with previous research by Deswanti (2021), which showed that the DP1342 isolate was indeed superior to the DP1341a isolate because it produced greater inhibitory effectiveness (55.98%) *in vitro*. However, both treatments were still considered less effective in controlling the growth of *A. flavus* on nutmeg seeds because the resulting infection inhibition percentages were only 38.67% and 28.00%. Although the two best inhibitory treatments were obtained, it appears that the percentage of infection inhibition produced by both isolates was less significant and decreased compared to the results of *in vitro* tests. This could be caused by several factors, such as yeast cells re-adapting to environmental conditions and nutrient sources in their new substrate (nutmeg seeds).

Despite their compatibility, the combination of DP1341a and DP1342 (KC-Consortium) did not produce greater inhibition of *A. flavus* compared to the single isolate DP1342 (Table 4). The KC-DP1342 treatment showed the highest inhibition (38.67%), outperforming both the consortium (28.00%), and DP1341a alone (21.33%). Similarly, in fermented filtrate treatments, the single isolates exhibited higher inhibition percentages than the combined filtrate, suggesting a neutral interaction rather than a synergistic effect.

This aligns with findings by Sadoudi et al. (2012), who observed neutral interactions between yeast isolates *Torulaspora delbrueckii* and *Saccharomyces cerevisiae*. The determination of optimal fermentation time is crucial for maximizing the antifungal compound production. DP1341a exhibited peak inhibition on day 10, while DP1342 peaked on day 14. These findings correspond to the stationary phase of yeast growth, a phase known for increased secondary metabolite production (Tanimura et al., 2016). The inhibition zone of benomyl, the positive control, was significantly higher (>31 mm), placing it in the 'very strong' inhibition category (Safitri et al., 2017).

Based on the data in Table 4, in general, the liquid culture treatment (KC-DP1341a; KC-DP1342; and KC-Consortium) produced greater inhibitory power against the growth of *A. flavus* with percentages of 21.33%, 38.67%, and 28.00%, respectively. This is different from the fermentation filtrate treatment (FHF-DP1341a; FHF-DP1342; and combined filtrate) which only produced inhibitory power percentages of 18.67%, 18.67%, and 16.00%, respectively. This is likely due to the antagonistic ability of the *Pseudozyma hubeiensis* yeast culture which, is dominated by competition for space and nutrients against *A. flavus*, so that it can be more inhibitive than the secondary metabolite products from the filtrate (Figures 3 & 4).

The average widest inhibition zone in the inoculum of isolates DP1341a and DP1342 on the 10^{th} and 14^{th} days after inoculation, with an average diameter of 12.5 and 6.3 mm, respectively. The average diameter results of the two isolates were included in the strong (12.5 mm) and moderate (6.3 mm) categories. Although *in vitro* results were promising, *in vivo* efficacy was moderate (inhibition zone 5–10 mm). Liquid cultures outperformed fermented filtrates, suggesting the importance of live yeast cells in exerting antagonistic effects. This result is supported by Sulaiman et al. (2021), who found that live yeast cultures were more effective than their filtrates in controlling *Colletotrichum acutatum*. The lower effectiveness of fermented filtrates may be due to reduced concentrations or degradation of antifungal metabolites.

Several mechanisms may contribute to the antagonistic effect of *P. hubeiensis*, including competition for space and nutrients, mycoparasitism, and biofilm formation. Previous studies have shown that *P. hubeiensis* produces hydrolytic enzymes such as chitinases, glucanases, and proteases, which degrade fungal cell walls (Chen et al., 2022). Moreover, the yeast can form biofilms on plant surfaces, enhancing colonization and resistance to environmental stress (Sharma et al., 2019).

Secondary metabolites such as volatile organic compounds (VOCs), siderophores, cellobiose lipids, and mannosylerythritol lipids (MELs) also play important roles in antifungal activity (Morita et al., 2013; Konishi & Makino, 2018). These compounds disrupt pathogen metabolism, compete for iron, and compromise membrane integrity, contributing to pathogen suppression.

The relatively lower *in vivo* effectiveness compared to *in vitro* may be attributed to several factors: adaptation challenges of yeast on nutmeg seeds, decreased cell viability due to storage

conditions, or increased virulence of *A. flavus*. As described by Casarica et al. (2016), pathogens may develop resistance to biocontrol agents through metabolic pathway shifts, enzyme modification, or reduced dependency on inhibited metabolites.

These results affirm the potential of *P. hubeiensis*, particularly DP1342, as a biological control agent. However, improvements in formulation, delivery methods, and environmental adaptation are needed to enhance its practical application. Future studies should explore microencapsulation, carrier-based systems, and synergy with other microbial antagonists for improved biocontrol performance.

CONCLUSION

The yeast isolate *P. hubeiensis* DP1342 demonstrates moderate *in vivo* antagonism against *A. flavus* and outperforms DP1341a and the consortium. However, efficacy drops *in vivo*, and further optimization is needed to develop an effective biofungicide formulation.

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