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Controlled release of *Michelia alba* oil vapour from plastic sachets to control the growth of *Aspergillus flavus* on brown rice and its possible mode of action

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Abstract

This study aimed to create antifungal volatile-releasing sachets, from various commercially available synthetics (Tyvek®; high density polyethylene; HDPE, polypropylene/polyethylene; PP/PE and polyamide/polyethylene; PA/PE) and bio-based plastic sachet materials (Polylactic acid; PLA and cellophane), containing *Michelia alba* (MA) essential oil to be used against *A. flavus* on malt extract agar (MEA) and brown rice. In addition, the bioactive compounds (total phenolic content and total flavonoid content) and antioxidant activity (DPPH, ABTS, and FRAP) of brown rice after treatment by the active sachets were examined. Results indicated that different sachet materials affected the release of the linalool and caryophyllene as antifungal volatiles. Tyvek® and PP/PE had a suitable permeability for controlling the release of the volatiles, which could be matched with the maximum concentration within 48 h, whereas PA/PE, PLA and cellophane had lower permeability. The antifungal volatiles released from Tyvek® and PP/PE sachets containing MA essential oil at 300 µl could completely inhibit the growth of *A. flavus* on MEA and brown rice for at least 20 days using accelerated conditions at 25 °C and 80%RH. No spore germination or deformed hyphae of *A. flavus* could be observed in the treated brown rice when compared to the control which showed swelling spores and regular structure with uniformity. In addition, the bioactive compounds and antioxidant activity of brown rice treated with MA essential oil in the Tyvek® and PP/PE sachets were higher than in the control. Therefore, this study demonstrates a good opportunity to implement antifungal volatile-releasing sachets containing MA for shelf-life extension and improving the antioxidant activity of brown rice.

Keywords: antifungal packaging; brown rice; *Michelia alba*; sachet materials; controlled release

1. Introduction

Brown rice has a high nutritional value and several beneficial bioactive compounds (e.g. phenolics, antioxidants, flavonoids and proanthocyanidin) (Zhou, Chen, Zhang, & Blanchard, 2014) thus, it has been recognised as a healthy food. Unfortunately, the highly-nutritional compounds in brown rice can induce and accelerate the growth of mould, particularly *Aspergillus flavus*, causing quality losses and safety issues and resulting in reduced shelf life (Castaño, Medina, & Magan, 2017). Therefore, the use of a safe, natural compound, such as an essential oil, to create an active packaging system for controlling the mould in brown rice is an interesting solution, which has recently been studied (Chaemsanit, Sukmas, Matan, & Matan., 2019; Songsamoe, Matan, & Matan, 2017). Essential oil has unique organoleptic properties that could either enhance or reduce the consumer acceptability of the products. To ensure the safety of brown rice while maintaining good flavour, *Michelia alba* (MA) essential oil is a possible solution, as it produces good sensory results when tested by consumers (Songsamoe et al., 2017; Songsamoe, Khunjan, & Matan, 2021).

Michelia alba is a medicinal plant that typically grows in Southeast Asia and has been used in traditional medicine and therapy for a long time (Asaruddin et al., 2003). Its extracts have many anti-inflammatory (Lee et al., 2005) and anti-cancer (Kumar et al., 2012) properties for the treatment of numerous diseases. Regarding the antifungal properties, Songsamoe et al. (2017) proved that the vapour phase of MA (300 µl L^{-1} air) could completely inhibit the spore germination and mycelium growth of *A. flavus*, and the combination of major and minor components (linalool and caryophyllene) at a specific ratio was the key factor for its antifungal effect. In addition, the vapour phase of MA has been successfully applied to inhibit mould

growth in brown rice and brown rice products for shelf-life extension and sensory-quality improvement. In terms of real application, the development of active packaging for brown rice may increase brown rice's consumption patterns for consumers around the world.

In active packaging systems, the sachet could be designed using plastic material and essential oil. The sachet would be placed inside the sealed packaging system. Sachet material is key to controlling the release of antimicrobial compounds from sachet to food for the purpose of the shelf-life extension. Furthermore, an active sachet is easily placed in any food container for commercial application (Petchwattana, Naknaen, Cha-Aim, Suksri, & Sanetuntikul, 2021). The sachet material used to overwrap absorbent material also affects the release rate and amount of antimicrobial volatile released into the packaging headspace. Various sealable materials were used to produce the sachet (e.g. semipermeable plastic films, porous non-woven fabrics and papers) (Otoni, Espitia, Avena-Bustillos, & McHugh, 2016). Synthetic plastic materials such as Tyvek®, PP laminated with PE (PP/PE) and PA laminated with PE (PA/PE) have been widely used as food packaging sachet materials for commercial purposes. In addition, bio-based plastic materials such as cellophane and PLA are of great interest to be used to minimise the use of synthetic plastic materials (Leelaphiwat, Auras, Burgess, Harte, & Chonhenchob, 2018). However, limited works have indicated the effect of these materials on the permeability of the antimicrobial volatiles of MA essential oil. Therefore, controlling the release of volatile components by synthetic (Tyvek®, PP/PE and PA/PE) and bio-based sachet materials (PLA and cellophane) for controlling the mould on brown rice was the main objective of this research. The mode of action for the controlled release of MA from

various types of materials against mould on brown rice was also investigated. The findings may be helpful for the commercial packaging of brown rice.

2. Materials and Methods

2.1. *Michelia alba* (MA), linalool and caryophyllene

MA derived by steam distillation, was provided by the Thai China Flavors & Fragrances Industry Co., Ltd., Bangkok, Thailand. Linalool and caryophyllene were purchased from Sigma–Aldrich (Darmstadt, Germany)

2.2. Plastic sachets

Commercial sealable synthetic and bio-based plastic film materials, including PP-laminated PE (PP/PE), PA-laminated PE (PA/PE), Tyvek®, PLA and cellophane were obtained from PackLAB, Università degli Studi di Milano, Milan, Italy. The thickness of plastic sachet materials was measured using a handheld digimatic micrometre calliper (Mitutoyo Corporation, Kanagawa, Japan). The average of a set of 20 measurements taken randomly across the surface of the material was calculated.

2.3 Brown rice

Brown rice (Thai Hom Mali Rice) was purchased from L H Rice International Co., Ltd., Nakronprathom, Thailand. Brown rice was surface sterilised using a UV lamp to ensure the removal of surface mould contaminants before testing.

2.4 Culture

A strain of *Aspergillus flavus* isolated from brown rice was obtained from the Research Center of Excellence in Innovation of Essential Oil at Walailak University in Nakhon Si Thammarat, Thailand. *A. flavus* was cultured on malt extract agar (MEA) before incubation at 25 °C for 7 days. The spore suspension was prepared by flooding 9 ml of sterile water into the agar slant before mixing. The number of viable spores was evaluated using the plate count method (10^8 cfu ml^{-1}).

2.5 Effect of antifungal volatiles released from different sachet materials on the growth of *A. flavus* on malt extract agar and brown rice

To produce the MA vapour-releasing sachet, plastic films (PP/PE, PA/ PE, Tyvek®, PLA and cellophane) were used to prepare the 6 × 6 cm sachet using the heating sealer (Medical H 460/610 Digital Accutemp, Gandus Saldatrici Co., Ltd., Milan, Italy). The absorbent material (Whatman filter paper No.1, Cytiva, Massachusetts, United States) containing MA at 300 µl was packed inside the sachets before they were placed inside the glass vessel (1 L).

The malt extract agar (MEA) plate and brown rice (100 g) inoculated with 1 ml of *A. flavus* spore (10^1-10^8) were also placed inside the vessel. Then, the vessel was immediately tightly sealed with a screw cap. Finally, all samples were incubated at 25 °C, 80%RH (Binder, BINDER GmbH co. ltd., Tuttlingen, Germany) for 20 days. The colony count of the growing mould was measured on day 5, day 10, day 15 and day 20 of the incubation period. The control was carried out in the same way, but using only the absorbent without MA in the sachet material. The reduction factor of spore germinated in MEA was calculated as Eq. 1. The percent of mould growth inhibition on the brown rice during storage was computed based on Eq. 2:

$$\text{Log}_{10}\text{ reduction} = \text{Log}_{10}(\text{Ac}) - \text{Log}_{10}(\text{At}) \quad \text{Eq. (1)}$$

$$\text{Growth inhibition (\%)} = [(\text{Log}_{10}(\text{Bc}) - \text{Log}_{10}(\text{Bt})) / \text{Log}_{10}\text{ Bc}] \times 100 \quad \text{Eq. (2)}$$

where Log_{10} (Ac) is the number of *A. flavus* colonies from the control plate

Log_{10} (At) is the number of *A. flavus* colonies from the treatment plate

Log_{10} (Bc) is the number of *A. flavus* colonies from the control brown rice

Log_{10} (Bt) is the number of *A. flavus* colonies from the treated brown rice

2.6 Microscopic analysis of *A. flavus* treated with MA in Tyvek® sachet

The inhibitory effect of MA in the Tyvek® sachet on the germination of *A. flavus* spores on MEA was determined. In brief, after the MEA solidified, 0.1 ml of spore suspension (10^8 cfu ml^{-1}) of *A. flavus* were added into the MEA and incubated at 25 °C with and without MA at 300 µl in the Tyvek® sachet in the vessel. After incubation for 12 and 24 h, conidial germination and germ tube elongation were observed by the compound microscope (Olympus CH30, Olympus Corp., Ltd., Tokyo, Japan).

To observe the growth of *A. flavus* on brown rice containing MA in the Tyvek® sachet, brown rice inoculated with the spore of *A. flavus* was incubated at 25 °C with and without MA at 300 µl in the Tyvek® sachet in the vessel for 20 days. To observe the effect of MA on the morphology of the spore and mycelium of *A. flavus* on brown rice, the spore and mycelium of *A. flavus* were added into brown rice for 7 days. Inoculated brown rice was then treated with MA at 300 µl. All samples were detached (⌀, approximately 1 cm) and air-dried. The dry brown rice was stuck to aluminium holders and sputtered with gold palladium at 20 mA for 120 sec. The morphology of *A. flavus* growing on the brown rice was also observed using a JEOL JSM-5800 LV scanning electron microscope (SEM) (JEOL, Ltd., Tokyo, Japan).

2.7 Release of volatiles of MA from different plastic sachet materials

The release kinetic of antifungal volatiles (linalool and caryophyllene) of MA from each sachet (PP/PE, PA/ PE, Tyvek®, PLA and cellophane) in the vessel (**Fig. 4a**) were identified using HS-SPME-GC-MS (PerkinElmer, Inc., Massachusetts, USA). A Perkin-Elmer Autosystem XL gas chromatograph equipped with a DB-5MS (30 m, 0.25 mm ID, a column with a film thickness of 0.25 µm), a Merlin Microseal™ Septum Kit installed on the Capillary Inlet system and a Turbomass mass spectrometer were utilised. The injection was conducted at 250 °C in splitless mode.

The GC programme was as follows: the initial temperature was 40 °C and held for 2 min. The temperature was then increased to 170 °C at a rate of 4 °C min^{-1} and sustained for half a minute. It was then further increased to 240 °C at a rate of 15 °C min^{-1} and maintained for an additional 2 min. Total run time was 43 min. The injector temperature was 250 °C. Helium was used as the carrier gas (flow rate of 1.2 ml min^{-1}). The mass spectrometer was operated in the electron impact mode (70 eV), and masses were scanned over an *m*/*z* range of 40–350 *m*/*z*. Compounds were identified by matching their mass spectra with the US National Institute of Standards and Technology (Gaithersburg, Maryland, USA) commercial library.

The concentrations of antifungal volatiles (linalool and caryophyllene) were determined using the standard curves for each compound. All experiments and analyses were carried out in triplicate. A transmission rate of volatile compounds (cm^3 m^{-2} 24 h^{-1}) was calculated, and their release curve was constructed.

2.8 Contact angle measurements

The affinity of the sachet (PP/PE, PA/ PE, Tyvek®, PLA and cellophane) for water, MA and its components (linalool and caryophyllene) was determined using contact angle measurements using an optical contact angle apparatus (OCA 15 Plus, Data Physics Instruments GmbH, Filderstadt, Germany) equipped with a high-resolution CCD camera. The sessile drop method was performed; 6 µl of the liquid sample was dropped onto the sachet surface with a precision syringe and allowed to equilibrate on the sample surface for 10 s. Using image processing and curve fitting, the contact angle between the baseline of the drop and the tangent at the drop boundary was determined. Each sample was measured for five replicates.

2.9 Effect of different sachet materials on bioactive compounds and antioxidant activity of brown rice

198 The total phenolic content and total flavonoid content as bioactive compounds
199 on the brown rice after exposure to MA at 300 µl sachet with Tyvek® or PP/PE were
200 measured. Total phenolic content was examined using Folin-Ciocalteu colourimetric
201 methods (Singleton, Orthofer, & Lamuela-Raventós, 1999) with some modifications.
202 The phenolic samples (250 µl) and distilled water (1 ml) were added to a test tube, and
203 then Folin-Ciocalteu reagent (250 µl) was added to react for 6 min. Then, a 2.5 ml 7%
204 sodium carbonate solution and 6 ml distilled water were added. The mixture was then
205 incubated at room temperature (30 ± 2 °C) for 90 min. The absorbance was measured
206 in a spectrophotometer (Thermo Scientific, Massachusetts, USA) at a 760 nm
207 wavelength. The results were reported as milligram gallic acid equivalents per g of the
208 sample (mg GAE g^{-1}). In addition, total flavonoid content was examined following the
209 method of Waewkum and Singthong (2021). The flavonoid sample (500 µl) was
210 mixed with 5% sodium nitrite (150 µl) and distilled water (2 ml). It was then
211 incubated at room temperature for 5 min. Afterwards, 150 µl of 10% aluminium
212 chloride hexahydrate solution was added to the sample and the mixture was incubated
213 for 6 min at room temperature. Then, 1 ml of sodium hydroxide (1 M) combined with
214 distilled water at a total volume of 5 ml was added. The solution was incubated at
215 room temperature for 10 min. The absorbance was measured at 510 nm, and the
216 results were reported as mg quercetin equivalent (mg QE) per g of sample.

217 For total antioxidant activity, 2,2–diphenyl–1–picrylhydrazyl (DPPH) and
218 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical-scavenging
219 abilities, and ferric reducing antioxidant power (FRAP) using the modified method of
220 Zeng et al. (2019) was employed with some modifications. A rice flour sample (0.2 g)
221 was extracted using methanol (10 ml). Then, it was shaken for 30 min. The
222 supernatant was collected using filter paper and centrifuged (ScanSpeed 1580 MGR,

LaboGene Co. Ltd., Lillerød, Denmark) at 4,500 × g for 10 min. The DPPH radical-scavenging ability was examined by mixing 450 µL of the sample with 4.5 ml of DPPH solution (0.3 mM). The mixture was incubated in darkness at room temperature for 30 min. After that, the absorbance was measured at 517 nm using a spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). For the ABTS assay, 100 µL of the sample was mixed with 3.9 ml of ABTS reagent and incubated in darkness at room temperature for 6 min, afterwards, the absorbance was measured at 734 nm. For the FRAP assay, 300 µL of the sample was mixed with 3 ml of FRAP reagent. Then, it was incubated at 37 °C for 8 min and the absorbance was measured at 593 nm. All results were also expressed as mg vitamin C equivalent per g of sample (mg VCEAC g sample^{-1}).

2.10 Statistical analysis

All results were expressed as the mean ± standard deviation. One-way analysis of variance and Duncan's post hoc test, with $p<0.05$ considered to be statistically significant, were applied in the statistical analysis conducted using Statistica software (StatSoft, Tulsa, Oklahoma, USA).

3. Results

3.1 Effect of different sachet materials on antifungal efficiency of MA in the vapour phase

The effect of antifungal volatiles released from different sachet materials on the growth of *A. flavus* on MEA is shown in **Fig. 1**. The volatile component of MA in Tyvek® and PP/PE sachet showed the highest effective antifungal activity on MEA and brown rice surfaces against *A. flavus* at 20 days of storage using accelerated conditions at 25 °C, 80%RH, followed by PA/PE. Conversely, the antifungal volatiles released from the cellophane and PLA sachets were not sufficient to inhibit mould

248 growth. In comparison with the control, full growth of *A. flavus* on MEA (~10^8 cfu
249 ml^{-1}) and brown rice (~10^6 cfu ml^{-1}) was found at days three and five, respectively.

250 **Fig. 2** shows the morphology of *A. flavus* growing on the MEA untreated and
251 treated with the sachet (Tyvek®) containing MA. The results illustrated that the mould
252 spores of *A. flavus* in the control swelled and germinated at the 12th h **(Fig. 2a)**. Then,
253 the hyphae elongated and developed to form a mycelium within 24 h in the sample
254 without MA vapour **(Fig. 2b),** whereas mould spores of *A. flavus* could not germinate
255 on agar under the sachet system containing MA vapour for 12 or 24 h **(Fig. 2c–d)**.

256 In addition, the SEM images **(Fig. 3)** illustrated the effect of MA in the sachet
257 (Tyvek®) on the morphology of spores of *A. flavus* at day 20 on brown rice. The
258 mould spores in the control could germinate and the mycelium and conidia could
259 develop and fully grown at day 20 (**Fig. 3a**). By contrast, *A. flavus* spores could not
260 germinate on the surface of brown rice at day 20 when using the Tyvek® systems
261 containing MA vapour **(Fig. 3b)**. To explain more about the mode of action of MA on
262 *A. flavus*, SEM showed the spore of the control treatment were aggregated with
263 uniformity, a regular structure **(Fig.3c)**. The treated spore sample showed smooth and
264 deformed conidiophores and could not germinate on the surface of treated brown rice
265 **(Fig.3d)**. Mycelium of *A. flavus* without MA **(Fig.3e)** demonstrated the full
266 development of *A. flavus* on the surface of brown rice, but treated with MA, damage
267 hyphae, create a wrinkled appearance of the mycelium, the rupture of membrane
268 integrity were confirmed **(Fig.3f).** These results conclusively confirm that the
269 antifungal volatiles released from the sachet containing the MA at 300 µl could stop
270 the spore germination of *A. flavus*. MA in the vapor phase inhibited *A. flavus* cells and
271 spores, which extended the shelf life of brown rice. Based on this result, MA in sachet

showed the potential for developing commercial antimicrobial packaging to control mold growth.

3.2 The release of kinetic and transmission rate of antifungal volatiles (linalool and caryophyllene) of MA

An examination of the chemical composition of MA in the vapour phase showed that linalool (70.22%) and caryophyllene (16.35%) were found to be a two major chemical compounds of MA in the vapour phase. Thus, this work demonstrated that both compounds can vaporise from an MA sachet into the air.

In addition, our previous published work (Songsamoe et al., 2017) demonstrated that the antifungal activity of MA at 300 µl L^{-1} air in the vapour phase comes from the collaboration of linalool (major component) and a small portion of caryophyllene (minor component) at the specific ratio of 10:1; however, the ratio of linalool and caryophyllene in this study when using Tyvek®, PP/PE, PA/PE, cellophane and PLA were 4.3:1, 5.5:1, 8.0:1.0, 1.0:0 and 1.0:0, respectively **(Table 1)**. The release of both volatile compounds from the Tyvek®, PP/PE sachet and their ratio in the air were emphasised in the present work with the result showing no mould growth. In addition, **Table 1** shows the thickness and volatile transmission rate of sachet materials. The results indicated that linalool and caryophyllene were released from the absorbent and could permeate through the synthetic-based sachet materials (Tyvek®, PP/PE and PA/PE) more than the bio-based sachet materials (e.g. PLA and cellophane).

The kinetic study of MA in different sachets is shown in **Fig. 4 (b–d)**. Tyvek® had no barrier property for linalool and had a minor barrier property for caryophyllene; the released volatiles could immediately permeate through the Tyvek® sachet into the headspace, and the concentration reached the equilibrium point within

297 6 h for linalool and 24 h for caryophyllene. That is a reasonable result because
298 Tyvek® (a brand of flashspun, high-density polyethene fibres) is a porous material;
299 thus, it has a good porosity property for the transmission of gases and volatile
300 compounds **(Fig. 4b-4c)**. PP/PE had a medium permeability for linalool and
301 caryophyllene. The released volatiles gradually permeated through the PP/PE sachet,
302 and the concentration reached the equilibrium point within 48 h for linalool and
303 caryophyllene **(Fig. 4b-4c)**. PA/PE had a lower permeability of volatiles. The released
304 volatiles could not reach the equilibrium point within 72 h. Conversely, the bio-based
305 materials had a very low permeability. Linalool could permeate through the
306 cellophane sachet into the headspace with a very low concentration; the concentration
307 reached equilibrium within 24 h **(Fig. 4d)**, whereas caryophyllene could not permeate
308 through the cellophane. Due to the release behaviour of linalool through the PLA
309 sachet, it seemed that an interaction existed between linalool and PLA, as the
310 concentration of linalool in the headspace decreased at the beginning and increased
311 again with time **(Fig. 4d)**. In addition, in terms of the ratio of linalool and
312 caryophyllene released through the sachets (**Table 1**), Tyvek® and PP/PE showed the
313 ratio to be fairly close to the normal ratio of linalool and caryophyllene in the vapour
314 phase of MA, whereas other materials showed a very different ratio.

315 **Table 2** shows the affinity between MA, linalool, caryophyllene, water and
316 sachet materials. The contact angle of water of all sachet materials was very high,
317 whereas the contact angle of MA and volatile compounds were lower. This indicated
318 that all sachet materials had hydrophobic surfaces. Tyvek® had the highest
319 hydrophobic property, creating the highest affinity with MA, linalool and
320 caryophyllene (ND), followed by PP/PE, PA/PE, PLA and cellophane, respectively.
321 Essential oils and their compounds naturally have a hydrophobic property; thus, MA

EO, linalool and caryophyllene showed a high affinity with the hydrophobic polymers, namely Tyvek® and PP/PE. Noticeably, given the permeability of linalool and caryophyllene through the sachet, high hydrophobic polymers (i.e. Tyvek® and PP/PE) showed higher volatile transmission rates than lower hydrophobic polymers (i.e. PA/PE, PLA and cellophane).

3.3 Bioactive compounds and antioxidant activity of treated brown rice

The total phenolic content of brown rice in the treated group (Tyvek® ~0.48 ± 0.02 mg GAE $g^{-1}$and PP/PE ~0.47 ± 0.02 GAE g^{-1}) was slightly higher than that of the control group (0.40 ± 0.03 GAE g^{-1}). Total flavonoids, significantly higher than the control (5.08 ± 0.43 mg QE g^{-1}), were observed in treated brown rice group (Tyvek® ~5.98 ± 0.57 mg QE g^{-1} and PP/PE ~5.68 ± 0.14 mg QE g^{-1}).

For antioxidant activity, the antioxidant capacity (i.e. DPPH, ABTS and FRAP) of the brown rice packed with Tyvek® and PP/PE sachet was higher than the control. Significant differences were observed between the DPPH values of control (0.74 ± 0.11 mg VCEAC g^{-1}) and treated groups (Tyvek® 1.07 ± 0.10 mg VCEAC g^{-1} and PP/PE sachet 1.09 ± 0.18 mg VCEAC g^{-1}). Significantly higher ABTS values were observed with the Tyvek® (3.42 ± 0.27 mg VCEAC g^{-1}) and PP/PE sachets (3.41 ± 0.13 mg VCEAC g^{-1}) than in the control 2.85 ± 0.13mg VCEAC g^{-1}. In addition, the FRAP value using the Tyvek® (1.27 ± 0.02 mg VCEAC g^{-1}) and PP/PE sachets (1.29 ± 0.01 mg VCEAC g^{-1}) increased when compared with the control (1.04 ± 0.06 mg VCEAC g^{-1}).

4. Discussion

The results from this study showed that linalool and caryophyllene were two major chemical compounds of MA in the vapour phase. Compared to the liquid phase of MA, this agreed with Suhem, Matan, Matan, Danworaphong, and Aewsiri (2017),

who reported that linalool (73.74%) and caryophyllene (7.35%) were found to be the main compounds of MA in the liquid phase. In addition, the results from this study demonstrated that different plastic sachets (PP/PE, PA/ PE, Tyvek®, PLA and cellophane) produced different levels of efficiency in reducing mould growth due to the release kinetic and transmission rates of MA components compound from inside plastic to the vessel. The Tyvek® sachet could instantly release the linalool and caryophyllene when placed in the system and reach an equilibrium in the shortest time, providing the correct ratio and concentration of the two volatiles to protect against mould growth. Similarly, the PP/PE sachet could gradually release both volatiles at a slower rate and reach an equilibrium at the same concentration necessary to inhibit mould growth. Conversely, other sachets (PA/PE, cellophane and PLA) could not release enough concentration and active ratio of both volatiles to inhibit mould. Due to the release behaviour of linalool through the PLA sachet, it seemed that an interaction existed between linalool and PLA, as the concentration of linalool in the headspace decreased at the beginning and increased again with time **(Fig. 4d)**. This result agreed with the work of Leelaphiwat et al., (2018), which found that linalool moved through the LDPE and PP film better than the PLA film.

The success of the Tyvek® and PP/PE sachets containing MA could be explained by the fact that the linalool and caryophyllene (antifungal volatiles) were released from the absorbent, penetrated through the sachet material and then diffused in the packaging headspace at a specific ratio. Some of the diffused antifungal volatiles in the headspace were then absorbed by the food matrix. The antifungal volatiles were continuously released from the sachet to compensate for the loss of volatiles in the air. The absorbed antifungal volatiles in the food matrix and the

diffused antifungal volatiles in the headspace were the important factors that collaborated to control mould growth in the food.

For the mode of action, the results from this study confirmed that the volatile MA from the Tyvek® and PP/PE sachet could inhibit the mould spore germination, but it has not been verified whether it could completely inactivate the mould spores. The mould spores might be active again if there is not a sufficient concentration of antifungal volatiles in the system. Therefore, controlling the concentration of antifungal volatiles in the headspace of the packaging during storage is essential for this system. This effect was similar to the antifungal activity of bergamot oil on the spore germination in the earlier report that limonene (major antifungal volatile of bergamot EO) at the minimum inhibitory concentration (MIC) may have affected the spore germination (Songsamoe, Matan, & Matan, 2016). In 2017, Basak and Guha also reported that betel leaf (*Piper betle* L.) could inactivate the spore germination of *A. flavus*. Furthermore, they found cytoplasmic coagulation, shrinkage, granulation and serious damage to the morphology of the treated spores. Hu, Zhang, Kong, Zhao, and Yang (2017) also confirmed that turmeric oil could inactivate the spore germination of *A. flavus* and create significantly rough walls on the spore surfaces. In addition, Sharma and Tripathi (2006) found that *Citrus sinensis* EO is extremely toxic to the spore germination of *A. niger*, however, the inactivation mechanisms of the mould spore germination of EO are still unclear. Some works mentioned that the antifungal components of EOs usually interact and penetrate through the cell membrane and can interrupt or denature the enzymes responsible for spore germination, energy production and synthesis of structural compounds, or that they interfere with the amino acid involved in germination (Carmo, Lima, & Souza, 2008). Therefore, in the present study, it might be possible that the antifungal volatiles could

affect some enzymes involved in spore germination resulting in an extension of the lag phase of spore germination.

This finding suggests that the synthetic plastic sachet (Tyvek® and PP/PE) had better permeability of antifungal volatiles when compared to bio-based plastic and could be developed for antimicrobial packaging to produce the antifungal volatile-releasing sachet and apply in food packaging, such as the brown rice packaging. In addition, the sachet could improve the antioxidant content of brown rice. It also shows an opportunity to apply the antimicrobial sachet releasing vapour of EO in food active packaging.

In addition, the results show that the volatile component released from the sachets could improve the bioactive compound and antioxidant activity of brown rice. This phenomenon might come from the effect of linalool and caryophyllene, which were released from the sachet and absorbed into the surface of the brown rice as agreed by Hu, Liu, and Deng (2020). This result also agreed with the study of Das, Singh, Chaudhari, Dwivedy, and Dubey (2021), which found that the linalool in rice during storage could improve the antioxidant ability of rice. Finally, this technique led to a significant reduction of lipid peroxidation in rice, without any adverse impact on organoleptic attributes.

Conclusions

Tyvek® and PP/PE had a suitable permeability for the production of the antifungal volatile-releasing sachet in brown rice packaging and were suitable for controlling the mould on brown rice. This sachet was sufficient to completely inhibit the spore germination of *A. flavus* on MEA and brown rice at 25 °C for at least 20 days. In addition, it improved the total phenolic, flavonoids and antioxidant content of brown rice packed with the sachet. Conversely, PA/PE, PLA and cellophane

sachets containing MA at the same concentration had lower permeability; thus, the release of linalool and caryophyllene could not reach the equilibrium within that period and could not control the mould growth. Therefore, the present study provided significant useful information for the production of an antifungal volatile-releasing sachet that contains EO in food packaging, particularly in brown rice packaging.

CRediT authorship contribution statement

Sumethee Songsamoe: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing – review & editing, Visualization, Project administration, Funding Acquisition. **Narumol Matan:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding Acquisition. **Luciano Piergiovanni:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Visualization, Supervision. **Sara Limbo:** Methodology, Investigation, Resources, Writing – review & editing, Visualization, Supervision.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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Figure legends

Fig. 1. Effect of antifungal volatiles released from different sachet materials containing *Michelia alba* oil at 300 µl on the growth of *A. flavus* on malt extract agar (a) and brown rice (b)

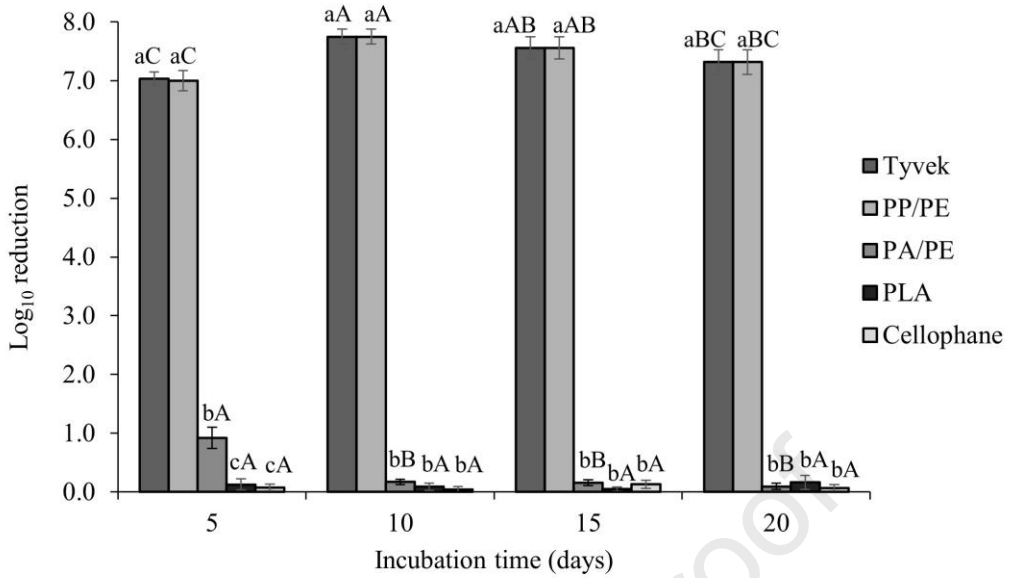
Error bars indicate standard deviation (S.D.). $^{a-c}$ Different superscripts letter are significantly different for each treatment in same day of incubation period ($p < 0.05$); $^{A-C}$ Different superscript capital letter are significantly different for each treatment in each day of incubation period ($p < 0.05$)

Fig. 2. Spore germination of *A. flavus* on malt extract agar (MEA) in the control group for 12 h (a) and 24 h (b) and in treated MEA with *Michelia alba* oil at 300 µl for 12 h (c) and 24 h (d)

Fig. 3. SEM images of *A. flavus* grown on brown rice in the control brown rice (a), and in the treated brown rice using Tyvek® containing *Michelia alba* oil (MA) on day 20 (b), spore of *A. flavus* without MA (c), and with MA (d), and mycelium of *A. flavus* without MA (e), and with MA (f)

"The red arrows indicated the abnormal morphology and surfaces of both of spore and mycelium of *A. flavus* after treatment with MA"

Fig. 4. The antifungal volatiles permeability through synthetic-based sachet material; linalool (a), caryophyllene (b) and through the bio-based sachet material; linalool (c)



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569 **(a)**

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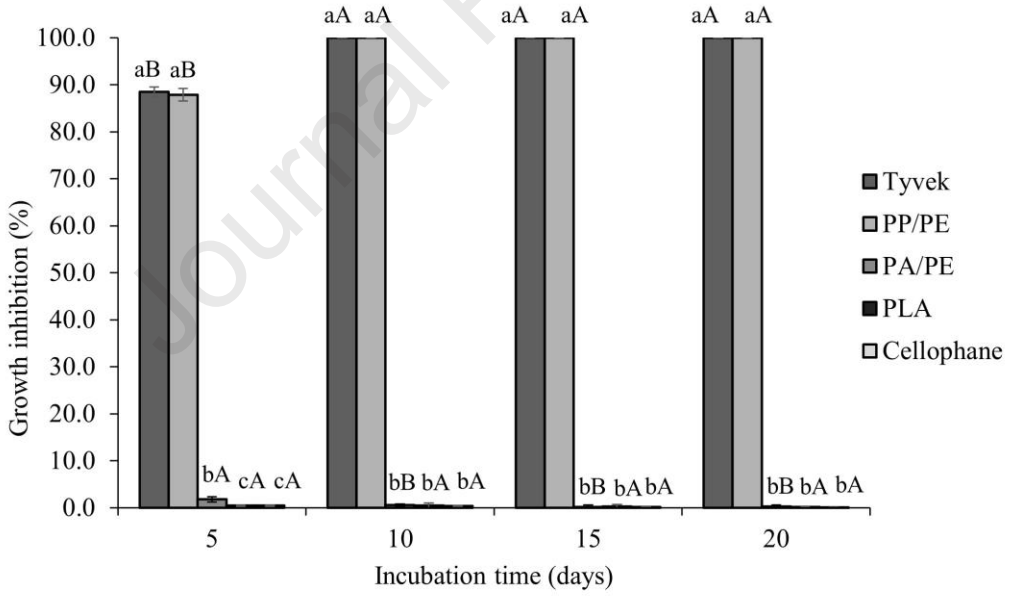

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572 **(b)**

573 **Fig. 1**

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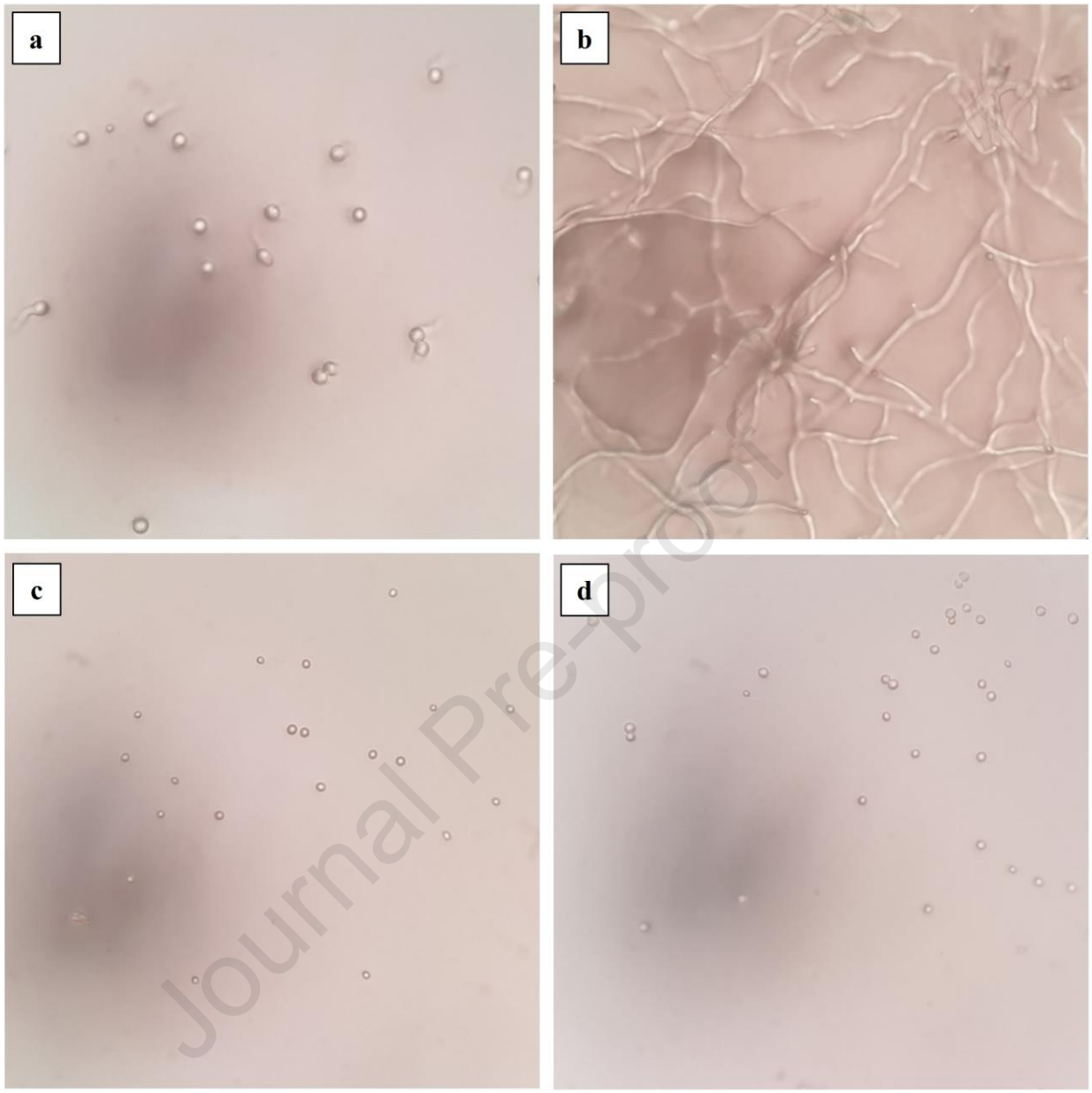

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578 **Fig. 2**

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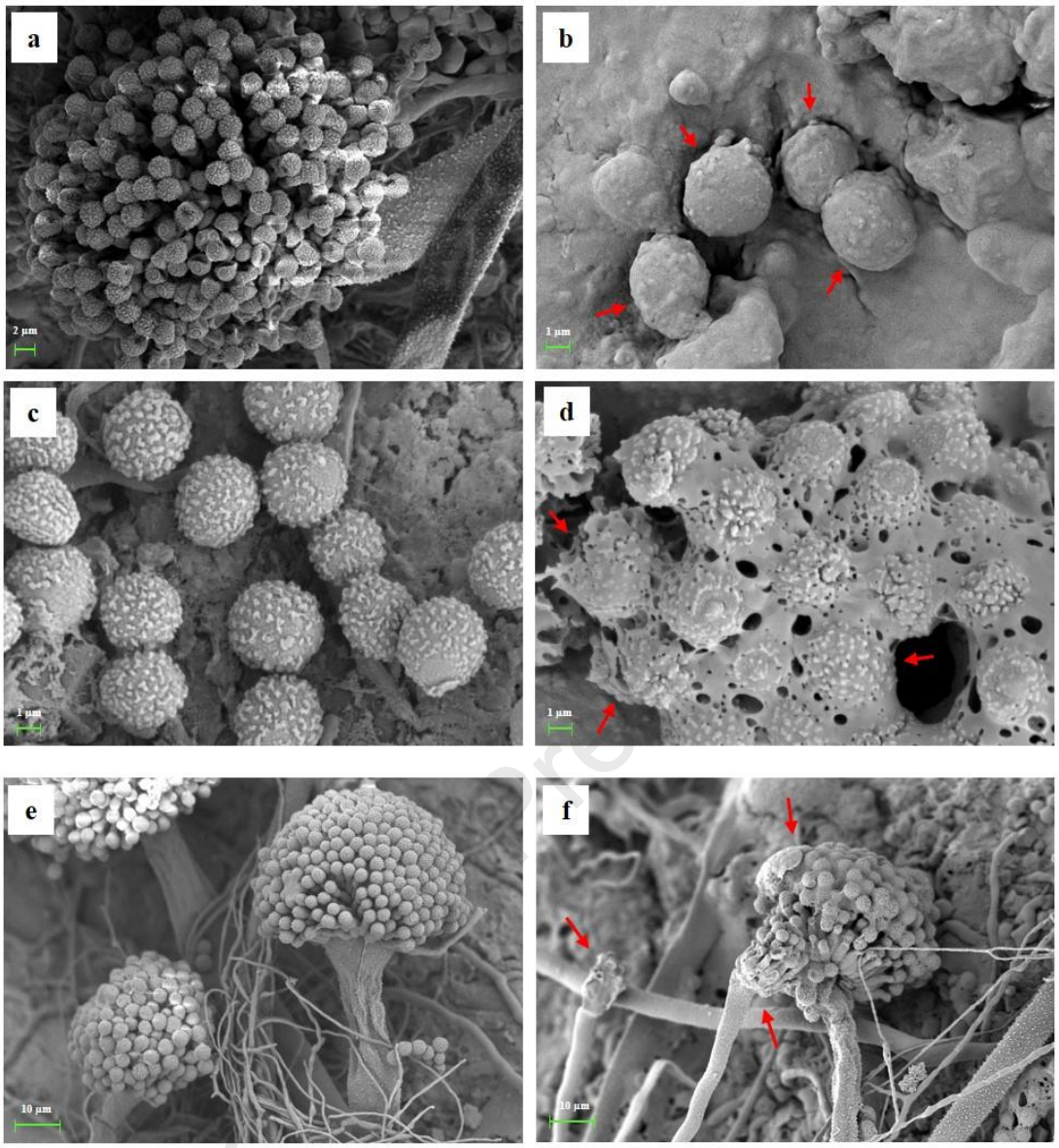
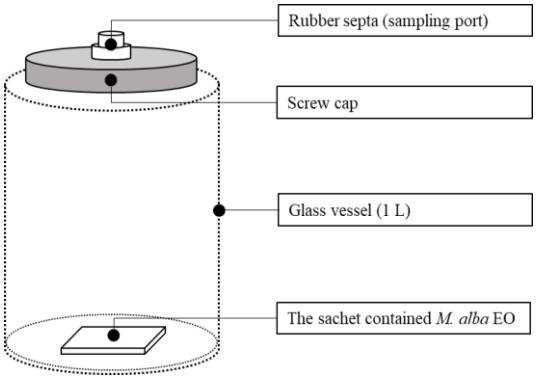
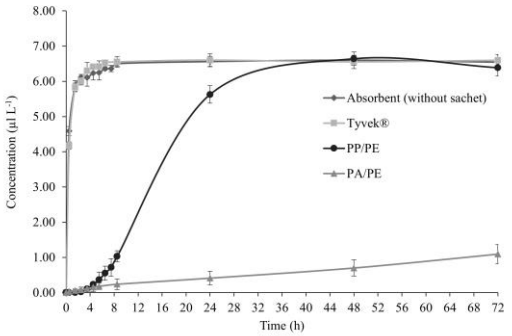


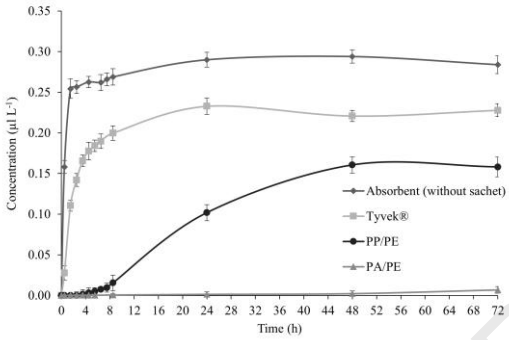
Fig. 3



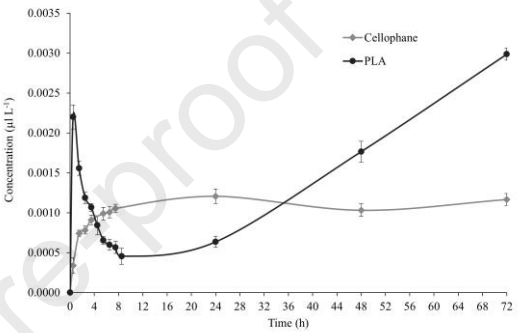
(a)



(b)



(c)



(d)

Fig. 4

610 **Table legends**

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612 **Table 1** The thickness and volatiles transmission rate of sachet materials and the ratio
613 of the antifungal volatiles of MA, which were released in the headspace (linalool and
614 caryophyllene) at 48 h.
615 [a–e] A different letter within a column is significantly different ($p<0.05$).

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617 **Table 2** MA, linalool, caryophyllene and water contact angles of sachet materials
618 *ND = not determined
619 [a–d] A different letter within a column is significantly different ($p<0.05$).

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635 **Table 1**

Material	Thickness (µm)	Ratio	Transmission rate (cm^3 m^{-2} 24 h^{-1})	
			Linalool	Caryophyllene
Tyvek®	168.2 ± 16.9	4.5:1.0	1.35500 ± 0.00707[a]	0.01600 ± 0.00282[a]
PP/PE	30.4 ± 1.9	5.5:1.0	1.13200 ± 0.00849[b]	0.00770 ± 0.00042[b]
PA/PE	65.0 ± 2.7	8.0:1.0	0.08600 ± 0.00566[c]	0.00016 ± 0.00001[c]
Cellophane	36.2 ± 1.3	1.0:0	0.00025 ± 0.00001[d]	0
PLA	35.0 ± 1.8	1.0:0	0.00013 ± 0.00001[e]	0

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Table 2

Material	***M. alba* oil**		**Linalool**		**Caryophyllene**		**Water**	
	Side 1	Side 2	Side 1	Side 2	Side 1	Side 2	Side 1	Side 2
Tyvek®	ND	ND	ND	ND	ND	ND	89.0 ± 2.5[a]	99.1 ± 1.1[a]
PP/PE	ND	5.2 ± 0.8[d]	ND	7.5 ± 0.9[c]	8.4 ± 0.6[c]	6.6 ±0.5[c]	80.6 ± 2.9[b]	93.5 ± 1.3[b]
PA/PE	11.1 ± 1.2[b]	8.0 ± 0.5[c]	10.6 ± 0.8[c]	8.5 ± 0.2[c]	4.4 ± 0.5[d]	8.2 ± 0.2[c]	75.0 ± 2.3[c]	90.8 ± 2.6[b]
PLA	12.3 ± 2.1[b]	12.9 ± 1.8[b]	20.7 ± 1.3[b]	19.4 ± 1.2[a]	15.3 ± 0.8[b]	12.0 ± 1.9[b]	74.1 ± 2.6[c]	78.1 ± 1.1[c]
Cellophane	27.1 ± 1.3[a]	16.1 ± 0.8[a]	26.8 ± 1.2[a]	15.1 ± 0.4[b]	36.6 ± 1.9[a]	18.8 ± 1.1[a]	60.4 ± 0.8[d]	91.2 ± 0.7[b]

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Highlights

- The synthetic plastic sachet containing MA could be developed for antimicrobial packaging.
- Tyvek® and PP/PE were suitable for controlling mould on brown rice up to 20 days (control 5 days)
- The synthetic plastic sachet had better permeability of antifungal volatiles when compared to bio-based plastic.
- Inhibition of mould spore germination was found to be the mode of the antifungal action of the sachets.
- The release of kinetic MA volatile from the plastic sachet was found to be the main factor to inhibit spore germination.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: