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Identification of Microbial Population during Oil Palm Frond (OPF) Composting using Light and Scanning Electron Microscopy

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ABSTRACT

This investigation highlights the activity and diversity of fungal population observed on oil palm fronds (OPF) during composting process employing Scanning Electron Microscope (SEM) and conventional identification methods. Composting is a controlled biological decomposition process, which converts organic wastes into humus-like material. It is a process that involves microbial degradation of complex organic materials under moist, self-heating and aerobic conditions; and characterized by a succession of various microbial populations. Two white rot fungi species were introduced as inoculant, namely *Trametes versicolor* and *Schizophyllum commune*. The main objective for inoculation was to shorten the composting period and produce high quality compost. In this study, the oil palm fronds (OPF) were composted for 14 weeks, with four treatment; i) control (untreated OPF), ii) OPF treated with *T. versicolor*, iii) OPF treated with *S. commune*, iv) OPF treated with both *T. versicolor* and *S. commune*, and four replicates for each. A total of eight genera of fungi namely *Aspergillus*, *Trichoderma*, *Absidia*, *Geotrichum*, *Trametes*, *Schizophyllum*, *Syncephalastrum* and *Beauveria* species were isolated and identified from composted OPF. Although *T. versicolor* and *S. commune* were introduced as accelerating agents, the presence of other fungal species perhaps occurred due to the indigenous microflora that already existed on OPF, leading to a succession of various fungi species based on the complexity of biological process in composting substrate.

Keywords: Composting, oil palm frond, white-rot fungi, inoculants, *Trametes versicolor*, *Schizophyllum commune*

ABSTRAK

Kajian ini menumpukan kepada aktiviti dan kepelbagaian populasi kulat yang terdapat pada pelepah sawit sepanjang tempoh pengkomposan, dengan menggunakan Mikroskop Elektron Pengimbas (SEM) dan teknik identifikasi konvensional. Pengkomposan merupakan proses penguraian biologi terkawal, yang menukarkan sisa organik kepada bahan-seperti-humus. Ia adalah suatu proses yang melibatkan penguraian mikrob terhadap bahan organik kompleks dalam keadaan lembap, pemanasan-diri dan aerobik; dan proses ini dicirikan oleh sesaran pelbagai jenis populasi mikrob. Dua spesies kulat pereput putih iaitu *Trametes versicolor* dan *Schizophyllum commune* telah diperkenalkan sebagai inokula dalam pengkomposan pelepah sawit. Objektif utama inokulasi adalah untuk memendekkan tempoh pengkomposan dan menghasilkan kompos berkualiti tinggi. Dalam kajian ini, pelepah sawit dikomposkan selama 14 minggu, dengan empat rawatan yang digunakan iaitu; i) kawalan (pelepah tidak dirawat), ii) pelepah dirawat dengan *T. versicolor*, iii) pelepah dirawat dengan *S. commune*, iv) pelepah dirawat dengan *T. versicolor* dan *S. commune*, dengan empat replikasi. Secara keseluruhannya, terdapat lapan genus kulat, iaitu *Aspergillus*, *Trichoderma*, *Absidia*, *Geotrichum*, *Trametes*, *Schizophyllum*, *Syncephalastrum* dan *Beauveria* telah diasingkan dan dikenalpasti dalam kompos pelepah sawit. Walaupun *T. versicolor* dan *S. commune* telah diperkenalkan sebagai agen pecutan, kehadiran pelbagai populasi kulat lain mungkin dipengaruhi oleh mikroflora semulajadi yang hadir dalam substrat pengkomposan, serta proses sesaran pelbagai spesies kulat yang bergantung kepada proses biologi kompleks yang berlaku dalam substrat pengkomposan.

Kata kunci: Pengkomposan, pelepah sawit, kulat pereput putih, inokula, *Trametes versicolor*, *Schizophyllum commune*

INTRODUCTION

Oil palm, *Elaeis guineensis* originated and grows wild in West Africa which led to its development as viable agricultural crop. The oil palm was introduced to Malaya in early 1870s by the British as an ornamental plant. Now, Malaysia's palm oil industry is the fourth largest contributor to the national economy and currently accounts for RM53 billion of the Gross National Income (GNI) per capita (NKEA, 2010). In 2009, palm oil industry claims to have established 4.7 million hectares of oil palm plantations, 416 mills, 43 crushers, 51 refineries, 18 oleochemical plants and 25 biodiesel plants in Malaysia (PEMANDU, 2010). The idea of oil palm waste composting arised from several aspects involving environmental concern, increasing level of waste disposal especially solid waste and

agricultural residues, scarcity of land disposal area and health hazard caused by mill and waste water effluents.

Noor *et al.* (2011) defined composting as a controlled biological decomposition process, which converts organic wastes into humus-like materials. Compost is an organic matter stabilized through biotransformation process (Acevedo *et al.*, 2005), which can also be used as starting material for a fresh batch of composting process (Nair and Okamitsu, 2010). According to Liu *et al.* (2011), composting is the microbial degradation of different organic materials under moist, self-heating and aerobic conditions; and this process is characterized by a succession of various microbial populations. Aerobic composting requires the availability of atmospheric oxygen during the period of decomposition, and characterized by high temperature, absence of foul odours and short stabilization. The high temperatures will destroy the weed seed and other pathogenic organisms. In contrast, anaerobic composting need longer stabilization time, but the advantages is that it needs minimum attention during the decomposition process (Deshmukh, 2010).

In composting process, different methods are adopted to improve the quality and maturity of compost produces, for example by using fungi and bacteria which are able to degrade the lignocellulolytic materials. Inoculums are the infective propagules of pathogen that are capable of causing infection or disease (Chaube and Pundhir, 2005). They are varied, from animal manures, decomposing material to the fungal and bacterial cultures. In composting, the adoption of multicellular fungi will induce the production of various hydrolytic enzymes that acts in synergistic ways to decompose lignocellulosic substrates. The decaying process of agriculture residues contribute significantly by lignocellulolytic enzymes secreted by fungi. Multicellular fungi have unique mechanism of degradation classified as white rot, brown rot and soft rot that preferentially degrade one or more wood components. White rot fungi acts either through simultaneous (nonselective) delignification or selective delignification (sequential decay) (Kubicek, 2012).

MATERIALS AND METHODS

Preparation of Composting Media

Pruned oil palm fronds (the petiole and leaflets) were obtained from a plantation in Batu 10, Jalan Sungai Batang, Sandakan, Sabah. These collected fronds were already half decomposed for about four weeks in between plantation. The inoculants of *Trametes versicolor* and *Schizophyllum commune* used in this study were obtained from the Department of Plant Science and Environmental Ecology, Faculty of Resource Science and Technology, UNIMAS, Sarawak. The fronds were manually shredded to reduce the size to approximately 3 to 5 cm for an efficient

microbial action during decomposition process. OPF were dried in oven (Memmert, Germany) at 30 °C for 24 hours and finely ground prior to further analysis. Fungal strains of *T. versicolor* and *S. commune* were cultivated on commercial potato dextrose agar (PDA, Merck) according to manufacturer instructions for strain characterization. Cultures were preserved at 4 ºC for short-term storage, sub-culturing was carried out in petri dishes contained PDA every 10 day interval.

Decomposition of OPF Waste (Laboratory Scale)

Shredded frond wastes (500 g on dry basis) were weighed and recorded as initial mass before further decomposed with selected fungi. The experiments were conducted in an open area with ambient temperature (27 to 30 °C), and laid-out in a white polystyrene box measuring 30 cm (length), 30 cm (width) and 15 cm (height) by using Complete Randomized Design (CRD). The treatments consisted of T1 - control (uninoculated frond), T2 - inoculated frond with *T. versicolor*, T3 - inoculated frond with *S. commune*, and T4 - co-inoculated frond with *T. versicolor* and *S. commune,* with 4 replicates each. The composting mixtures were left for 14 weeks. Turning was done intermittently to mix the outer and inner parts of compost. Water was sprayed to maintain the moisture level in composting materials.

Method of Inoculation on OPF Substrate

The fungal inoculated agar block (1 cm x 1 cm) were cut-off and directly transferred to OPF substrate for inoculating purposes. Inoculations were done every two week interval, for the first six weeks of composting (Haddadin *et al.*, 2009). The other technique used was microbial suspension method, in which portion of fungal agar blocks were transferred to 250 mL conical flask with 100 mL distilled water. The contents were agitated for 1 hour and left for a while before they were poured into composting materials (Zeng *et al*, 2009; Wang *et al.*, 2011).

Microbial Analysis

Throughout the composting period, the microbial examinations on compost were carried out by two approaches; scanning electron microscope (SEM) and conventional isolation and identification methods.

Scanning Electron Microscope (SEM)

At the end of composting process, ultrastructure micrographs of composting materials were obtained by using scanning electron microscope (SEM) (JSM-5610 LV JEOL, Japan) as proposed by Manoch *et al.*, 2008. For SEM study, several pieces of OPF were dissected (5x5 mm^2) carefully. The samples with fungal spores

were placed on the aluminum stub by using the carbon tape. They were coated with gold and examined under SEM operating at 10 kV. The resulted electron micrographs highlighted the microscopic structures of inoculants and substrate selected, and the incorporation level of *T. versicolor* and *S. commune* with OPF.

Isolation and Identification of Microorganisms in Compost

The isolation and identification technique of fungi were used as described by Molla *et al.* (2002) with slight modification. Microorganisms present in composting materials were isolated before further identification using compound microscope fitted with camera (Nikon Eclipse EZ200). The direct inoculation method was adapted where portions of compost material were placed into the PDA petri dish. The plates were then incubated at ambient temperature (27 to 30 °C), for 4 to 5 days for fungi growth. Occurrences of the fungi were recorded. The hyphal tips of each morphologically different mycelium that emerged from the plated tissues were transferred again onto a new petri dish containing PDA to get pure culture and for identification. The cultures were incubated at room temperature. A small amount of mycelium was taken from the fungi colony and was placed on microscopic slide with the aid of a scalpel. A drop of lactophenol blue, which was used as stain, was then dispensed onto the microscopic slide. A cover slide was gently placed onto the microscopic slide. The fungi that have been isolated were observed under the compound-microscope and the stereo-microscope. The identification was made on the basis of conventional cultural and morphological characteristics. Microscopic examination of fungi growth isolated from compost was examined by observing the colonial morphology: colour of colony, texture, shape and surface appearance; cultural characteristics: asexual and sexual reproductive structures such as sporangia, conidial head, arthrospores, septate or non-septate vegetative mycelia. All the observations were recorded for further reference. The species identifications were done based on detail descriptions and resembling images provided by Cannon and Kirk (2007) and Watanabe (2010).

RESULTS AND DISCUSSION

Scanning Electron Micrograph (SEM)

The scanning electron microscope (SEM) was used to analyse the ultrastructure of the inoculants and OPF. SEM micrograph illustrates the sporulation of inoculated fungi in samples which cannot be seen by naked eyes, ultrastructure of visible colonies, and the proliferation of fungal hyphae with composting substrate within inner and outside areas of the inoculation point. In this section, the SEM micrograph and ultrastructure morphology of composted OPF will be described

briefly. Figures 1, 2, 3, 4 and 5 illustrate the ultrastructure electron micrograph of OPF, uninoculated OPF (control), single inoculation of OPF with *T. versicolor*, single inoculation of OPF with *S. commune* and co-inoculation of OPF with *T. versicolor* and *S. commune*, respectively.

This microscopic structure shows the cross sectional view of oil palm fibrous strands. These parenchyma tissues were occupied with perforated pores that were distributed on frond strands (Fig. 1).

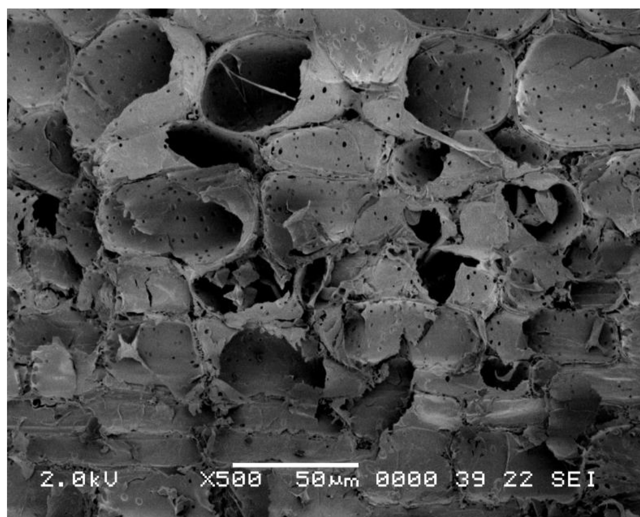

Fig. 1. Ultrastructure electron micrograph of oil palm frond (OPF) that was used as the composting substrate.

The spores and mycelia from fungi were detected on the surface of OPF in uninoculated OPF (Control). The mycelial networks were scattered within the vascular fiber. Although there were no inoculants added, the fungal growth indicated the presence of natural microflora in the OPF compost (Fig. 2).

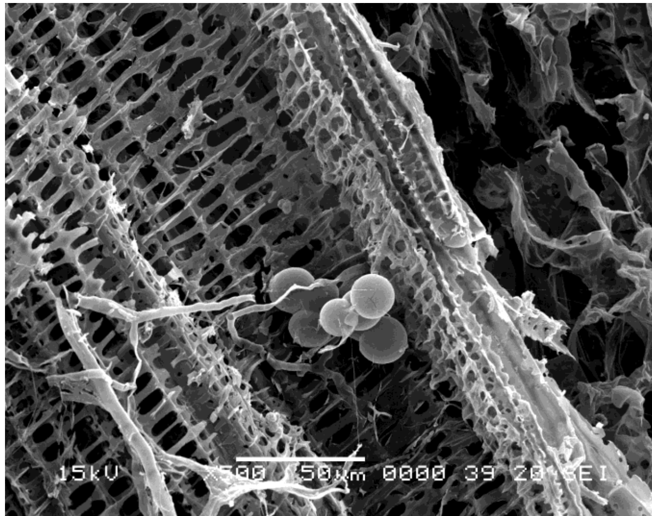

Fig. 2. Ultrastructure electron micrograph of uninoculated OPF (Control).

The clustered spores were attached on fibrous strand, with the silica bodies present over the strand surface. The silica bodies were deposited on circular craters with perforated bottom (Fig. 3).

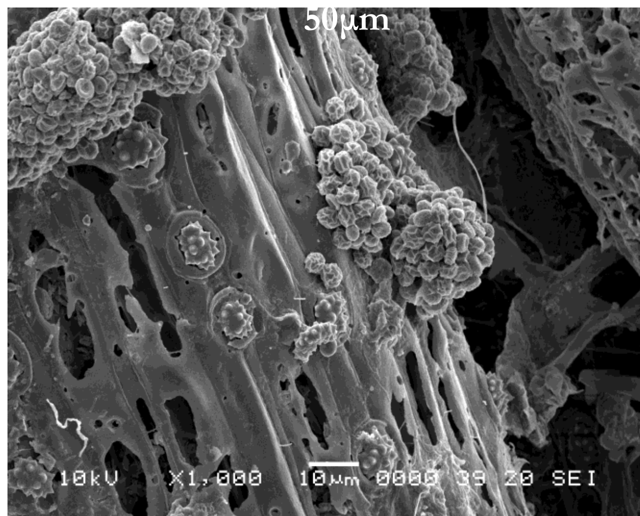

Fig. 3. Ultrastructure electron micrograph of OPF inoculated with *Trametes versicolor*.

The mycelial networks covered the entire surfaces of fibrous strand, and these mycelia were attached to the globule (Fig. 4)

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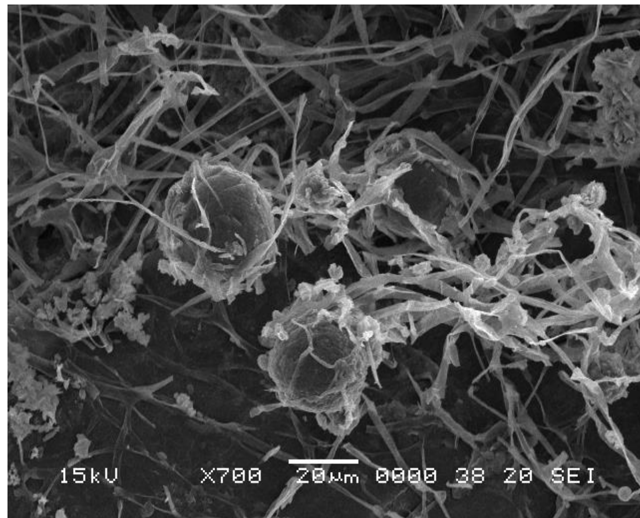

Fig. 4. Ultrastructure electron micrograph of OPF inoculated with *Schizophyllum commune*.

The withered spores clumped together on fibrous strand. These spores thrived well at suitable conditions for fungal survival (Fig. 5).

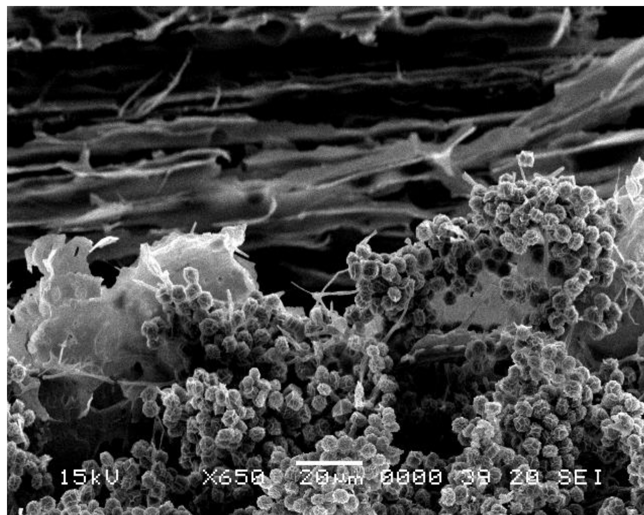

Fig. 5. Ultrastructure electron micrograph of co-inoculation of OPF with *Trametes versicolor* and *Schizophyllum commune*.

Isolation and Identification of Microorganisms in Compost

The microbial population during the composting process was studied in order to understand the effect of inoculation on composting system. The succession of fungi species on composted oil palm frond (OPF) could be observed during 14 weeks of composting process. A total of 36 filamentous fungal strains representing eight genera: *Aspergillus*, *Trichoderma*, *Absidia*, *Geotrichum*, *Trametes, Schizophyllum*, *Syncephalastrum* and *Beauveria* were isolated from composted fronds. Table 1 summarizes the characteristics of fungi isolated from composted OPF with brief descriptions.

Table 1. List and identification of 36 isolated filamentous fungi from the composted OPF.

Source of isolation	Macroscopic characteristics	Identification of species
Stage I: Middle of composting process Culture media: Potato dextrose agar (PDA)		
OPF Control No. 1-2-1	Cloud-like, covered entire petri dish in 6-7 days. Soft, whitish grey protruding web colonized in petri dish.	*Absidia* sp.
OPF Control No. 1-1	Whitish grey colony with thread like branching filaments (hyphae). Dominated ½ of entire petri plate.	*Syncephalastrum* sp.
OPF TV No. 5-1	Whitish-grey colony, protruding web with cloud-like structure, covered entire petri dish in 6-7 days.	*Absidia* sp.
OPF SC No. 10-1	Greyish-brown, compact dusty-like structures, with fuzzy edges. Covered ¼ part of entire PDA culture in 5 days.	*Trichoderma* sp.
OPF TV SC No. 15-1	White, hyaline with serrated periphery. Initially, white, dry to cottony colonies but became yeast-like or slimy when disturbed on the surface.	*Geotrichum* sp.
OPF TV SC No. 16-1	Greyish-brown, compact dusty-like structures, with fuzzy edges. Covered ¼ part of entire PDA culture in 5 days. Reverse (bottom side) showed bright yellow colour.	*Aspergillus* sp. (*A. niger*)

Table 1. List and identification of 36 isolated filamentous fungi from the composted OPF (continued)

Source of isolation	Macroscopic characteristics	Identification of species
Stage II: End of composting process Culture media: Potato dextrose agar (PDA)		
OPF Control No. 4-2-1	Colonies are fast growing, loose cottony aerial mycelium, white at first becoming pale grey with age, and up to 1.5 cm high.	*Absidia* sp.
OPF Control No. 1-2	Black, powdery texture. Covered ¼ of petri dish after 21 days.	*Aspergillus* sp. (*A. niger*)
OPF Control No. 2-2	Black, powdery texture with compact spore mats.	*Aspergillus* sp. (*A. niger*)
OPF Control No. 3-2	Pinkish and velvety texture, ½ colony growth from central region extended to periphery.	*Beauveria* sp.
OPF Control No. 4-2	Olive green to tan, velvety colonies; fully colonized in 21 days. Developed in centric ring from central region extended to periphery.	*Trichoderma* sp.
OPF Control No. 5-2	Tan, powdery colonies; developed in centric ring from central region extended to periphery. 3/4 colonization in PDA plate after 21 days.	*Aspergillus* sp.
OPF Control No. 5-3	Black, small dust-like structures covered the entire petri dish. Full colonize in 4-5 days.	*Aspergillus* sp. (*A. niger*)
OPF TV No. 9-1	White to light yellow colony, fully colonize PDA culture by 5-6 days. Hyphae without reproductive structures.	*Trametes* sp. (*T. versicolor*)
OPF TV No. 6-1	White colonies on central to periphery. Thread like septate mycelium without any reproductive structure.	*Trametes* sp. (*T. versicolor*)
OPF TV No. 6-2	Dark green colony, compact texture; grow in distance extended to periphery.	*Trichoderma* sp.
OPF TV No. 7-2	Black, powdery with plenty of loose spores. Covered whole petri dish after 21 days	*Aspergillus* sp. (*A. niger*)
OPF TV No. 8-2	Dark green colony, velvety and compact texture. Fully colonized the entire PDA plate in 21 days.	Unknown sp.

Table 1. List and identification of 36 isolated filamentous fungi from the composted OPF (continued)

Source of isolation	Macroscopic characteristics	Identification of species
Stage II: End of composting process Culture media: Potato dextrose agar (PDA)		
OPF TV No. 9-2	Whitish to light olive appearance, cottony texture; vigorous growth only at the central region.	Unknown sp.
OPF TV No. 10-2	Dry, black, powdery with plenty of loose spores covered on PDA culture. Colonized whole petri dish after 21 days	*Aspergillus* sp. (*A. niger*)
OPF TV No. 6-3	Black mould with dense mycelia mat. Covered entire PDA petri dish in 6-7 days. Branched with septate hyphae.	*Aspergillus* sp. (*A. niger*)
OPF TV No. 7-3	Fluffy white and green colony. Reverse yellow.	Unknown sp.
OPF TV No. 8-3	Branched & septate hyphae, black mould with dense mycelia mat. Covered entire PDA petri dish in 6-7 days.	*Aspergillus* sp. (*A. niger*)
OPF SC No. 13-1	White to light grey colony, fully colonize PDA culture by 5-6 days.	*Schizophyllum* sp.
OPF SC No. 11-2	Black, powdery with plenty of loose spores on media culture. Colonized whole petri dish after 21 days	*Aspergillus* sp. (*A. niger*)
OPF SC No. 12-2	White, thread-like appearance at the central region.	*Syncephalastrum* sp.
OPF SC No. 13-2	White to dark green colony, velvety, grows vigorously at the central region. Colonize ¼ of entire PDA plate after 2 weeks.	Unknown sp.
OPF TV SC No. 18-1	Whitish and fluffy colonies, growth from central to periphery of PDA media.	*Schizophyllum* sp.
OPF TV SC No. 16-2	Green, velvety texture; scattered in circle from central region to periphery. Reverse with greenish-white colour.	Unknown sp.
OPF TV SC No. 17-2	Green, dry and velvety texture; colonized the entire PDA plate. Reverse with light yellow colour.	Unknown sp.

Table 1. List and identification of 36 isolated filamentous fungi from the composted OPF (continued)

Source of isolation	Macroscopic characteristics	Identification of species
Stage II: End of composting process Culture media: Potato dextrose agar (PDA)		
OPF TV SC No. 18-2	Black and brownish powdery with plenty of loose spores on media culture; colonized whole petri dish after 21 days.	*Aspergillus* sp. (*A. niger*)
OPF TV SC No. 19-2	Black, dry, powdery with plenty of loose spores on media culture. Colonized entire region in centric ring formation. Reverse with magenta-red colour.	*Aspergillus* sp. (*A. niger*)
OPF TV SC No. 20-2	Black, powdery with plenty of loose spores colonized 1/3 of PDA plate after 21 days.	*Aspergillus* sp. (*A. niger*)
OPF TV SC No. 16-3	Whitish aerial growth projected to the surface of PDA plate; grow at periphery region.	*Absidia* sp.
OPF TV SC No. 17-3	Black, small dust-like structures covered the entire petri dish. Full colonize in 4-5 days.	*Aspergillus* sp. (*A. niger*)
OPF TV SC No. 18-3	Whitish aerial growth projected to the surface of PDA plate; colonized ½ of PDA plate.	*Absidia* sp.

Although *T. versicolor* and *S. commune* were introduced as accelerating agents, the presence of various fungi species on the composted OPF could be distinguished. The fungi were either naturally existed (indigenous microflora) on OPF or developed under suitable condition based on the complexity of biological process in composting substrate. The microbial growth depends on environmental and nutritional conditions of composting materials (Vargas-García *et al.*, 2005). Regardless of the introduced inoculants, different fungi populations begin to emerge as the composting progressed. During the whole composting period, the *Aspergillus* sp. became the most prevalent as their populations were present frequently during the isolation process. This observation was similar with Ashraf *et al.* (2007) study, in which they found that of all isolates, members of genus *Aspergillus* were most dominant (38%) in agricultural and kitchen waste composts. As predicted, the successions of fungi in compost were influenced by temperature, moisture content and the OPF substrate composition.

Diversity of Fungi in Composted OPF

Aspergillus sp. especially *A. niger* grows in black colony that consisted of dense conidiophores. The conidiophores stipe is smooth-walled, hyaline or pigmented. Conidial heads are radiate, with brown and sub-spherical conidia (Kushwaha, 2004). Under the microscope, the dark black mycelia mass were scattered on the PDA culture. The abundance of *A. niger* in compost is due to its widespread distribution on decaying vegetation, organic materials and soil (Ashraf *et al.*, 2007). Colonies of *Geotrichum* sp. expanded rapidly with flat or cottony aerial mycelium. Initially, *Geotrichum* sp. was white, dry to cottony colonies but became yeast-like or slimy when disturbed on the surface. The colonies had somewhat fimbriate margin with serrated periphery (Kushwaha, 2004). The arthroconidia are unicellular, in chains, subglobose to cylindrical smooth, resulting from the fragmentation of undifferentiated hyphae by fission through double septa. Later, these structures will develop into septate mycelium (either rectangular in shape or rounded at the ends). Colonies of *Absidia* sp. shows white to greyish colour, and mycelium profusely branched with stolons and rhizoids. Colonies were fast growing, loose cottony aerial mycelium, white at first then becoming pale grey with age, and up to 1.5 cm high. Under microscope observation, the sporangiophores were hyaline to faintly pigmented, with simple or sometimes branched-arising solitarily from the stolons, in groups of three, or in whorls of up to seven. Colonies of *Trichoderma* sp. grows rapidly, at first white and downy, later developing yellowish-green to deep green compact colonies. The conidia are subspherical, hyaline and appeared green in mass (Kushwaha, 2004). Conidia developed with smooth or rough walls and were formed in slimy conidial heads (gloiospore) clustered at the tips of the phialides. In study by Ashraf *et al.* (2007), *Trichoderma* sp. was isolated from substrate with lignocellulose sources, such as bark, sawdust and wood chips.

On PDA media, *Schizophyllum* sp. (Basidiomycetes) colonies were spreading, woolly, pale greyish-brown, and their macroscopic fruiting bodies were formed in concentric zones. Hyphal system is monomitic, hyaline and often with clamp connections (Cannon and Kirk, 2007). Colonies of *Trametes* sp. showed fluffy white texture on PDA media. Their spores are 4-6 x 1.5-2.5 μm, slightly curved-cylindrical, smooth, hyaline, white to pale yellow in deposit. *T. versicolor* is able to degrade lignin through non-selective delignification (Tuomela *et al.*, 2000). Colonies of *Syncephalastrum* sp. (Zygomycota) were dense, have rapid growth with cottony texture. On PDA media, their colony appeared white to grey with aerial mycelium. Soon, they became dark grey with the development of sporangia. Sporangiophores were stolon-like, branched with terminal vesicles bearing merosporangia directly over their entire surface. Sporangiophores are simple, commonly sympodial or racemose branched (Cannon and Kirk, 2007). The genus *Beauveria* (Ascomycota) have moderate growth rate with cottony to powdery texture. They appeared white at first and later becoming yellowish white to pinkish on PDA media. The reverse

was white or pale. Genus *Beauveria* is characterised by the sympodial development of single-celled conidia (ameroconidia) on a geniculate or zig-zag rachis (Kushwaha, 2004). Microscopically, hyphae were hyaline and septate with smooth- and thin-walled, loose or sometimes fasciculate. The conidia (diameter: 2-4 μm) are hyaline, globose to ovoid in shape, one-celled with smooth and thin-walled (Kushwaha, 2004).

Justification of Inoculating Agents in Composting Process

In this study, the introduced inoculants played a significant role in reducing the mass volume of composted OPF when compared to uninoculated treatment, at the end of composting period. At the end of 14 weeks composting period, the maximum volume reduction of 62.8% was recorded in OPF treated with *S. commune*, whereas least volume reduction of 50.4% was recorded in the Control (uninoculated OPF). Treatments of T2 (inoculation of *T. versicolor*) and T4 (co-inoculation of *T. versicolor* and *S. commune*) recorded volume reduction of 57.5%, and 54.3%, respectively. This might be due to the ability of inoculants to compete with natural microflora and survive within the compost pile. Oviasogie *et al.* (2010) emphasized that fungi inoculant was found to speed up composting process, and able to produce mature compost in six weeks. In that circumstance, inoculation was done by mixing approximately 10% of mature compost into fresh composting mixture, not by isolating the spores. Kabbashi *et al.* (2006) listed four filamentous fungi of *Phanerochaete chrysosporium*, *Trichoderma harzianum*, *Aspergillus niger* and *Penicillium* sp. that have the potential as inoculating agent in bioconversion of agro-industrial waste. Xi *et al.* (2012) mentioned that inoculation significantly improve composting efficiency by improving the degradation rate of aliphatics, proteins and polysaccharides; beside increasing the humification degree in compost. In efficient composting, for the microorganism to be selected as inoculant, it is important to evaluate their adaptation to the environment and the size of inoculum applied (Vargas-García *et al.*, 2005). In composting, it was justified that the characteristics of raw materials and inoculants will determine the effectiveness of inoculation itself (Vargas-García *et al.*, 2007). Xi *et al.* (2012) also suggested that organic matter composition and temperature should be considered so that the objective of inoculation be achieved.

In case of control treatment, Vargas-García *et al.* (2005) claimed that a lack of inoculation of composting substrates may attribute to the competitiveness of indigenous microbiota; therefore able to speed up the substrate decomposition process. Dashtban *et al.* (2009) stated that in natural environment, lignocellulosic residues are degraded by multiple co-existing lignocellulolytic microorganisms. Therefore, conversion of lignocellulosic components can be done by co-culturing two or more compatible fungi which are able to utilize that material. Chi *et al.* (2007) concluded that in biopulping, the interspecific reaction among white rot fungi must be well understood as they may possibly shorten the incubation time.

The potential fungi to be used in co-culturing systems include *Pleurotus ostreatus*, *Ceriporiopsis subvermispora* and *Physisporinus rivulosus* (Chi *et al.*, 2007). Feng *et al.* (2011) suggested that it is reasonable to use ligninolytic enzymes in order to enhance lignocellulosic waste composting. The addition of enzyme in composting could enhance the microbial activities in system (Feng *et al.*, 2011). Based on established field trial by Piškur *et al.* (2011), it was mentioned that the biological activity of inoculum is indicated by the increase in temperature and relative humidity in between the space of wood chips used.

Most of the fungi isolated in this study was particularly from the mesophilic taxa since the heap temperature did not even reach the thermophilic phase (>45 °C). Ashraf *et al.* (2007) found that fungal species are numerous during both mesophilic and thermophilic phases of composting. The fungi either with white, grey or green fuzzy growth or unseen filaments are obvious on compost outer layer, when the temperature is high (Ashraf *et al.*, 2007). Mesophilic fungi present as the compost warm up moderately (below 40 °C); this saprophytic fungi being able to utilise cellulose and hemicellulose but not as good as thermophilic fungi (Panda and Hota, 2008). These populations of saprophytic fungi, at least one of them, significantly contributed to the humification process with their ability to degrade major polymer components of cellulose, hemicellulose and lignin (van Heerden *et al.*, 2002). It is therefore justified that since only OPF substrate were utilized without any activator applied, the populations of fungi in this case were not diversified when compared to previous reports. Ashraf *et al.* (2007) suggested that combination of substrate helps to enhance the diversity of saprophytic microorganisms and increases the variety of microorganism for degradation purposes; in consideration that different substrate composition would harbour different population of fungi under favourable conditions.

There were several studies on the significance of using inoculums in composting. Studies by previous researchers (Faure and Deschamps, 1990; Nair and Okamitsu, 2010), considered the use of microorganism's inoculant only as a strategy since there is no significant difference of inoculated and uninoculated microbial on composting material. According to Schuchardt (2005), the addition of inoculum for the composting process is not necessary, because high number of microorganisms present in the waste and of their short generation time. The contradictory results should not be surprising, in consideration of the complexity of chemical and biological processes that occurs in different types of composting substrate and other factors related to composting process. Vargas-García (2006) explained that one of the most influential factors is the raw material used for composting since the qualitative and quantitative chemical composition of the substrate, and microbiological activity influenced by it. Zeng *et al.* (2009) stated that the microbial species used as inoculant as well as inoculating time, should also be considered as important factors in composting process.

CONCLUSION

In this study, OPF provides a suitable substrate for composting process. It was found that single inoculation of either *T. versicolor* or *S commune* definitely will assist in degrading the OPF substrate to a large degree with their hydrolytic enzymes. Compared to other treatments, single inoculation of *S. commune* indicated higher percentage of volume reduction, with a value of 62.8%. Therefore, single inoculation of *S. commune* was suggested in composting of OPF. After 14 weeks of composting, the composted OPF has merely become mature as the heaps temperature stabilized to ambient temperature. Resulting compost was brown in colour with homogeneous appearance, and no unpleasant odour detected. As a conclusion, the adoption of inoculants to fasten the natural process or obtaining high quality final compost is still not fully understood, since composting involved complex biological activities depending on substrate used and other related parameters. Nevertheless, further studies are required to search for suitable species or strain of microorganisms specific to OPF as substrate, and to enhance the composting process in order to produce high quality compost.

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