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PHYTOTOXICITY OF OIL PALM RESIDUES USED AS MULCH FOR LETTUCE, TOMATO, CUCUMBER AND AMARANTH SEEDLINGS

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Wastes of the Malaysian oil palm industry included empty fruit bunch (EFB), palm oil mill effluent (POME), oil palm frond (OPF), chopped trunk (OPT), palm pressed mesocarp fibre (PPMF) and palm kernel shell (PKS), amounting to173.02 million tonnes in 2014. Utilization of these residues is important to maximize uses of resources and overcome environmental pollution. Raw EFB and PPMF have been widely used in oil palm plantation, vegetable farming and landscape industry, as mulching and organic fertilizer. OPT and OPF are produced from old palm and left to rot in the field. Fresh plant wastes could release phytotoxic compounds, affecting growth of other plants. Therefore, the objective of the study were (1) to determine the phytotoxic effect of oil palm wastes, EFB, PPMF, OPF and OPT, using seedling bioassay and seed germination test on four vegetable: lettuce (*Lactuca sativa),* tomato *(Solanum lycopersicum),* cucumber *(Cucumis sativus)* and green amaranth (*Amaranthus viridis)*, and (2) to identify phenolic acid phytotoxic compounds in extracts of selected raw oil palm wastes using thin layer chromatography (TLC). In Experiment 1, oil palm wastes were water extracted to produce aqueous extracts of OPT, EFB, PPMF and OPF. The application OPF aqueous extracts caused greatest radicle length reduction of lettuce, tomato, cucumber and green amaranth seedlings by61.9%, 65%, 53% and 52.6%, respectively, compared with control. PPMF and EFB extracts reduced radicle length significantly compared with OPT extracts, but degree of inhibition on radicle length treated with PPMF was higher than EFB aqueous extracts, with more than 20% radicle reduction for all seedlings, except cucumber seedlings, compared with control. However, OPT aqueous extracts showed no inhibition in radicle growth, hypocotyl growth and fresh and dry weights of all seedlings and total seed germination percentage and mean germination time. Thus, OPT extracts did not release any phytotoxic compounds and it can be concluded that OPF released phytotoxic compounds and degree of inhibition was higher compared with other wastes. PPMF inhibited seedling growth, with greater inhibition compared with EFB and OPT extracts. In Experiment 2, OPF and PPMF were further extracted using four solvents, hexane, diethyl ether, chloroform and ethyl acetate, to determine presence of phytotoxic compounds using seedling bioassay and seed germination test of lettuce,

tomato, cucumber and green amaranth. OPF diethyl ether extract exhibited highest inhibition on radicle length of lettuce (48.3%) and tomato (62.6%), but lower for cucumber (27.9%) and green amaranth (26.4%) seedlings. The OPF diethyl ether extract also reduced hypocotyl length of lettuce and green amaranth seedlings significantly compared to control. PPMF diethyl ether extract also reduced radicle length of lettuce, cucumber and green amaranth and hypocotyl length of green amaranth seedlings compared to control. Both solvent extracts showed high inhibition of radicle and hypocotyl length of seedlings compared with other treatments. Phenolic acid compounds present in OPF diethyl ether and PPMF diethyl ether extracts were identified using TLC and compared with 10 phenolic acid standards OPF extracts contained 4-hydroxybenzoic acid and syringic acid, while PPMF diethyl ether extracts contain 4-hydroxybenzaldehyde and syringic acid. OPF and PPMF contained phytotoxic compounds affecting plant growth and their utilization as mulching materials should be avoided to ensure that plants grow without inhibition. However, availability of OPT for mulching is limited since it is only available during oil palm replanting season. Thus, utilization of other readily available and safe oil palm wastes is essential to ensure that seedlings growth is not affected with mulching materials applied. EFB is one of the waste products of oil palm that would be safe to use and easily obtained since it has lower toxicity on seedling growth compared with OPF and PPMF wastes.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

KEFITOTOKSIKAN DALAM SISA KELAPA SAWIT SEBAGAI SUNGKUPAN BAGI ANAK POKOK SALAD, TOMATO, TIMUN DAN BENIH AMARANTH

Oleh

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Sisa daripada industry kelapa sawit Malaysia termasuk tandan kosong (EFB), efluen kilang minyak sawit (POME), pelepah kelapa sawit (OPF), batang cincang (OPT), gentian mesokarpa kelapa yang ditekan (PPMF) dan kulit isi rong sawit (PKS) berjumlah 173.02 juta tan metric pada tahun 2014. Penggunaan sisa-sisa ini adalah penting untuk memaksimumkan penggunaan sumber dan mengatasi pencemaran alam sekitar. EFB dan PPMF mentah telah digunakan secara meluas di ladang kelapa sawit, sayur-sayuran dan industry landskap, sebagai sungkupan dan baja organik. OPT dan OPF dihasilkan daripada kelapa tua dan dibiarkan mereput di lapangan. Sisa tumbuhan segar boleh melepaskan sebatian fitotoksik sehingga menjejaskan pertumbuhan tumbuhan lain. Oleh itu, objektif kajian ini adalah (1) untuk menentukan kesan fitotoksik sisa kelapa sawit, EFB, PPMF, OPF dan OPT, menggunakan bioesei anak benih dan ujian percambahan benih kepada empat sayur-sayuran: salad (*Lactuca sativa*), tomato (*Solanum lycopersicum*), timun (*Cucumis sativus*) dan amaranth hijau (*Amaranthus viridis*), dan (2) untuk mengenal pasti sebatian fitotoksik asid fenolik dalam ekstrak sisa kelapa sawit mentah terpilih menggunakan kromatografi lapisan nipis (KLN). Dalam Eksperimen 1, sisa kelapa sawit adalah ekstrak akueus OPT, EFB, PPMF dan OPF. Aplikasi ekstrak akueus OPF menyebabkan pengurangan panjang radikel tertinggi bagi benih salad, tomato, timun dan amaranth hijau sebanyak 61.9%, 65%, 53% dan 52.6% masing-masing, berbanding dengan kawalan. PPMF dan ekstrak EFB mengurangkan panjang radikal dengan ketara berbanding dengan ekstrak OPT, tetapi tahap perencatan panjang radikal yang dirawat dengan PPMF adalah lebih tinggi daripada ekstrak akueus EFB, lebih daripada 20% pengurangan radikel untuk semua benih, kecuali anak benih timun, berbanding dengan kawalan. Walaubagaimanapun, ekstrak akueus OPT tidak menunjukkan perencatan pertumbuhan radikel, pertumbuhan hipokotil dan berat segar dan kering untuk semua benih dan jumlah peratusan percambahan benih dan purata masa percambahan. Oleh itu, ekstrak OPT tidak mengeluarkan sebarang sebatian fitotoksik, dan ia boleh membuat kesimpulan bahawa OPF mengeluarkan sebatian fitotoksik dan tahap perencatan adalah lebih tinggi berbanding dengan sisa lain. PPMF menghalang pertumbuhan anak benih, dengan

perencatan yang lebih besar berbanding dengan ekstrak EFB dan OPT. Dalam Eksperimen 2, OPF dan PPMF telah diekstrak menggunakan empat pelarut, heksana, dietileter, kloroform dan etil asetat, untuk menentukan kehadiran sebatian fitotoksik menggunakan bioesei anak benih dan benih ujian percambahan salad, tomato, timun dan amaranth hijau. Ekstrak dietileter OPF menunjukkan perencatan tertinggi bagi panjang radikal anak benih salad (48.3%) dan tomato (62.6%), tetapi lebih rendah untuk timun (27.9%) dan amaranth hijau (26.4%). Ekstrak dietileter OPF juga mengurangkan panjang hipokotil anak benih salad dan amaranth hijau dengan ketara berbanding dengan kawalan. Ekstrak dietileter PPMF juga mengurangkan panjang radikal anak benih salad, timun dan amaranth hijau dan panjang hipokotil anak benih amaranth hijau berbanding kawalan. Kedua-dua ekstrak pelarut menunjukkan perencatan radikel dan panjang hipokotil anak benih yang tinggi berbanding dengan rawatan lain. Sebatian asid fenolik di dalam ekstrak dietileter OPF dan PPMF telah dikenalpasti menggunakan KLN dan dibandingkan dengan 10 standard asid fenolik. Ekstrak OPF mengandungi asid 4-hidroksibenzoik dan asid siringik, manakala ekstrak dietileter PPMF mengandungi 4-hidroksibenzaldehid dan asid siringik. OPF dan PPMF mengandungi sebatian fitotoksik yang menjejaskan pertumbuhan tumbuhan dan penggunaannya sebagai bahan sungkupan perlu dielakkan untuk memastikan bahawa tumbuhan dapat tumbuh tanpa perencatan. Walaubagaimanapun, Ketersediaan OPT sebagai sungkupan adalah terhad kerana iahanya boleh didapati ketika musim penanaman semula kelapa sawit. Oleh itu, penggunaan bahan buangan kelapa sawit lain yang sedia ada dan selamat adalah penting untuk memastikan bahawa pertumbuhan anak pokok tidak terjejas dengan bahan-bahan sungkupan yang digunakan. EFB adalah salah satu daripada bahan buangan kelapa sawit yang selamat untuk digunakan dan mudah diperolehi kerana ia mempunyai ketoksikan yang lebih rendah pada pertumbuhan anak benih berbanding dengan sisa OPF dan PPMF.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

EFB	Empty fruit bunch
OPF	Oil palm fiber
PPMF	Palm press mesocarp fiber
OPT	Oil palm trunk
TLC	Thin layer chromatography
ANOVA	Analysis of variance
g	Gram
mm	Millimeter
cm	Centimeter
mg	Milligram
DMRT	Duncan multiple range test
LSD	Least significant difference
POME	Palm oil mill effluent
PKS	Palm kernel shell
FFB	Fresh fruit bunch
K	Potassium
N	Nitrogen
Mg	Magnesium
P	Phosphorous
C	Carbon
m	Meter
℃	Degree Celsius
%	Percentage
g/L	Gram per liter
Mm	Millimeter
EC	Electrical conductivity
mS cm^{-1}	Millisiemens per centimeter
SAS	Statistical Analysis System
UPM	Universiti Putra Malaysia
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
OECD	Organization for Economic Coorperation and Development
kg	Kilogram
m/v	Mass per volume
rpm	Revolutions per minute
DNA	Deoxyribonucleic acid
NH_3	Ammonia

CHAPTER 1

GENERAL INTRODUCTION

Oil palm (*Elaeis guineensis Jacq*.), in the family of Arecaceae is considered as the largest plantation industry in Malaysia and one of the major contributions towards the economic growth in some developing countries. It is cultivated in about 43 countries with over 12 million hectare in 2009. In Malaysia, the area under cultivation was increased by 5 times from 4 decades ago (Teoh, 2010). This is due to the suitable environment with high temperature between 22 to 33°C, high rainfall (>2000 mm) and sufficient sunlight (>7 hours) for these crops to grow (Tiquia *et al*, 1996). According to the Malaysian Palm Oil Board, about 45% of total palm oil world production comes from Malaysia and 52.1% of total world population consumed palm oil (Anon., 2014). The oil palm industry produces broad ranges of product from crude oil to cosmetics product (Mohammad *et al*., 2012). Besides some of oil palm wastes have been burnt in boiler to produce steam for electricity generation used in the palm oil mill industry (Sheil *et al*., 2009).

However, the increasing oil palm production has released abundant agricultural residues in Malaysia. There are several type of residues produced from oil palm industry which are empty fruit bunches (EFB), palm oil mill effluent (POME), oil palm fronds (OPF), chopped trunks (OPT), palm pressed mesocarp fibre (PPMF) and palm kernel shell (PKS) (Basiron and Weng, 2004). Thus, it is advisable that waste products of these industry be investigated with the objective of using the raw material for other industry to get desired product (Singh *et al.,* 2011). Therefore, the utilization of these residues is important to maximize the uses of resources and to overcome environmental pollution (Ahmad, 2001). Many researches were carried out to support the possibilities of converting oil palm residues into many value added products such as EFB and POME which have been used as organic fertilizer and mulch in oil palm areas, while pressed fibre and shell are used as a fuel to generate steam and energy used for the operation of the mill. Other than that, palm kernel is effectively used to produce palm kernel cake for residual livestock food (Ahmad, 2001).

Besides that, raw EFB and PPMF have been widely used in vegetable farming and landscape industry as mulching and organic fertilizer (Yusof, 2006). However, fresh plant wastes used for organic fertilizer and mulching need to decompose to avoid the harmful accumulation of lipids and volatile compounds that can inhibit plants growth. Besides, decomposition is essential to redu00ce phytotoxin compound (Zucconi *et al*, 1981).

Phytotoxicity is the negative effects of substances arising from decomposing crop residues on growth and yield of the crops. The phytotoxicity of plant residue has been associated with the presence of various organic compounds including water soluble phenolic compounds that widely distributed all over the part of various plants species (Rice, 1984). Application of POME to seedling is proven to inhibit the growth of seedling significantly. The inhibitory effect of raw POME on plant growth is similar to the phytotoxicity exhibited by other types of crop residues (Radziah *et al*,1997). However the degree of phytotoxicity depends on the types of crop residues, decomposition period and the sensitivity of plant root system toward phytotoxic compound. There are several type of compounds was classified as phytotoxic such as phenolic acid, heavy metal, ammonia, excessive accumulation of salt, lipid and organic acid (Radziah *et al*, 1997). However, phenolic acid is commonly identified as phytotoxin and inhibit growth of seedling and reduce crop yield (Einhellig, 1985)

In Malaysia, there are a lot of vegetable farms present in high or lowland. One of the most important planting materials that are highly demanded in vegetable production is mulching material, compost and organic fertilizer. Oil palm wastes such as EFB and OPMF, can be used as mulching material in vegetable production (Yusof, 2006) while OPF commonly been put under oil palm tree between the row to act as mulching and conserve soil structure (Mohammad *et* al., 2012). A study from Kim and Rahman, (2002) proved that mulching with EFB improved the soil exchangeable potassium, calcium and magnesium and pH. Mulching material gave many advantages to the plant growth such as increases in soil organic matter content, improves soil structure, improves soil water retention and increases cation exchange capacity (Kim and Rahman, 2002). Abdul Mutalib *et al.,* (2009) showed that EFB wastes in combination with inorganic fertilizer could support shoot growth of cabbage. However, the information of phytotoxity effect on different type of oil palm wastes is still not available except for POME.

A series of seed germination and seedling bioassay tests were undertaken on 4 selected vegetable seedlings; *Amaranthus viridis* (green amaranth), *Lactuca sativa* (lettuce), *Solanum lycopersicum* (tomato) and *Cucumis sativus* (cucumber);

- To determine the phytotoxic effect of four different oil palm wastes, empty fruit bunch (EFB), palm pressed mesocarp fiber (PPMF), oil palm frond (OPF) and oil palm trunk (OPT).
- To identify the phenolic acid phytotoxic compounds that present in extracts of selected raw oil palm waste by using thin layer chromatography (TLC).

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