International Journal of Agriculture, Environment and Bioresearch

Vol. 2, No. 04; 2017

ISSN: 2456-8643

GROWTH RESPONSE OF VEGETABLE SEEDLING RADICLES TO PHYTOTOXIC COMPOUNDS IN OIL PALM WASTES

Mohd Zakwan Zamri[1], Nor Elliza Tajidin[1],Siti Hajar Ahmad[1]and Rosenani Abu Bakar[2]

1Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia,
43400 UPM, Serdang, Selangor, Malaysia.

2Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia,
43400 UPM, Serdang, Selangor, Malaysia.

ABSTRACT

Oil palm residues have been widely used as mulching materials for production of vegetables. Palm oil mill effluent (POME) and oil palm fronds (OPF) were reported to contain allelochemical compounds that suppress growth of seedlings. Therefore, a study was conducted to determine the phytotoxicity effects of different types of oil palm wastes [chopped oil palm trunks (OPT), empty fruit bunches (EFB), palm pressed mesocarp fibre (PPMF) andOPF], by conducting seedling bioassay and seed germination tests on four selected vegetable species, lettuce (Lactuca sativa), tomato (Solanum lycopersicum), cucumber (Cucumis sativus) and green amaranth (Amaranthus viridis). The application of OPF aqueous extract gave the highest reduction in radicle length for the lettuce, tomato, cucumber and green amaranth seedlings. Also, PPMF and EFB extracts reduced radicle length significantly compared to OPT extract. However, the OPT aqueous extract showed no inhibition in radicle and hypocotyl growth, fresh and dry weights, total seed germination percentage and mean germination time for all seedlings. These results indicated that OPT did not release any phytotoxic compounds while OPF released phytotoxic compounds and the degree of inhibitions were higher compared with other wastes. Meanwhile, PPMF also inhibited seedling growth, with greater inhibition compared with the EFB and OPT extracts.

Keywords: seedling bioassay, seed germination, oil palm fronds, aqueous extract

Introduction

Oil palmis considered as the largest commodity that has helped to change the scenario of agriculture and economy in Malaysia. In 2014, about10.8 million tonnes of palm oil were produced on 5.39 million hectares of total planted area, thus, making Malaysia as the second largest palm oil producer (39%) and exporter (44%) in the world(Abdullah and Sulaiman, 2013; MPOB, 2014). Despite the obvious benefits toward the economic growth, the oil palm industry also generates 53 million tonnes of oil palm waste residues including oil palm fronds (OPF), empty fruit bunches (EFB), chopped oil palm trunks (OPT), palm pressed mesocarp fibre

(PPMF) and palm kernel shelland palm oil mill effluent palm (POME)(Ali and Abbas, 2006; Abdullah and Sulaiman, 2013).

Among the oil palm waste residues, EFB and PPMF have been widely utilized as mulching and organic fertilizer in the oil palm plantations, vegetable farms and landscape sites (Kerdsuwan and Laohalidanond, 2011; Suhaimi and Ong, 2001). OPF, usually created during the harvesting process, is dumped off on rowsin between plants to control weeds and return nutrients back to the soil (Mohammad et al., 2012). Researches have been carried out to support the possibilities of converting oil palm residues, such as EFB and POME, into many value added products such as organic fertilizers and mulching materials in oil palm areas, while pressed fibres and shells are used as a fuel to generate steam and energy in oil palm mill operations. Also, palm kernel residuals are effectively used to produce palm kernel cake for livestock food (Ahmad, 2001).

Applications of fresh agriculture wastes as mulching materials can cause phytotoxic effects on horticultural plants. Commonly, during the decomposition of organic waste materials, phytotoxic compounds would be released which could influence the growth of seedlings. The effect of the compounds could differ between crop species. Bogatek et al. (2006) found that the application of aqueous extract of sunflower leaf on mustard seedlings using bioassay test showed that the fresh and dry weights were reduced when treated with certain concentration (10% m/v) of the aqueous extract. For oil palm wastes, Dilipkumar et al. (2015) reported that OPF, including rachis and leaflet, have the capacity to suppress seedling growth of *Eleusine indica* (L.) Gaertn. The authors also stated that OPF contained high level of allelochemicals that lead to a significant reduction in seedling emergence of *Eleusine indica* (L.) Gaertn. Radziah et al. (1997) reported that POME has been shown to contain phytotoxic compounds that affect radicle elongation of tomato seedlings, whereas for other oil palm wastes, the phytotoxic effect is still unknown.

Therefore, the objective of this study was to determine the phytotoxic effects of four types of oil palm wastes (OPT, EFB, PPMF, and OPF) by conducting seedling bioassays and seed germination tests on four selected vegetable species, leaf lettuce *(Lactuca sativa)*, tomato *(Solanum lycopersicum)*, cucumber *(Cucumis sativus)* and green amaranth *(Amaranthus viridis)*.

Materials and Methods

Sample collection

Two of the oil palm wastes, OPT and OPF, were obtained from an oil palm plantation at Universiti Putra Malaysia (UPM), Serdang, Selangor while the other two types, EFB and PPMF, were obtained from an oil palm factory in Sepang, Selangor, Malaysia. The wastes were put into sacks and transported to the Faculty of Agriculture, UPM and stored at 13 ^oC before further processing. The wastes were shredded to about 8 cm by using a wood chipper (74950, Bear Cat, USA) washed with tap water to remove soil, fungus and dust particles. After that, the wastes were air dried under a plastic-shelterhouse for 4 days before being ground using a grinder and sieved using a 2-mm mesh.

Twenty grams of each sample were taken randomly, and then mixed with 100 mL distilled water and vigorously shaken for 6 hours using an orbital shaker (SK-300, Lab Companion, UK). The suspension was transferred into a clean centrifuge tube and centrifuged at 1000 revolution per minute (rpm) for 20 minutes. The fine particulate in the aqueous extract was removed by using a Whatman filter paper No. 4 followed by filter papers No 1 and 42, sequentially. The phytotoxicity of the filtrates was determined using the seed bioassay.

Bioassay test

Four type of vegetables, leaf lettuce, tomato, cucumber and green amaranth were selected for the seedling bioassay tests. These four species were selected because they are common test plants used for phytotoxicity test (Wang, 2000; Bhowmik, 2003). For the seedling bioassay, the degree of toxicity of the aqueous extract was evaluated by measuring the radicle length, hypocotyl length, radicle diameter, and fresh and dry weights of the seedlings. The seed bioassay test was determined using the germination percentage, and fresh and dry weights of the germinated seeds with radicle lengths of 2 mm.For each vegetable species, 10 seeds per replication were taken randomly and then continually washed for three times with distilled water. Then, the seeds were germinated in sterile plastic petri dishes (90 × 15mm) lined with wet cotton and allowed to germinate in the dark at ambient temperature (±25 °C). Uniformly germinated seeds from each replication and treatment with radicle length of 2 mm were taken for the seedling bioassay.

ISSN: 2456-8643

Two millilitres of each oil palm waste aqueous extracts were poured into separate sterile plastic petri dishes (90 mm × 10 mm) lined with a single layer of sterilised Whitman No.1 filter paper. A 2 mL sample of sterilised distilled water was used as the control treatment. Ten germinated seeds of each species were placed onto separated petri dishes. Each petri dish was sealed using parafilm to avoid surface water evaporation and kept in the dark at ambient temperature. After three days of treatment application, seedling growth test was carried out by measuring radicle and hypocotyl lengths, radicle diameter, and fresh and dry weights. Seed bioassay was conducted by determining the total seed germination rate and average number of days for seeds to germinate. Both the tests were carried out in the Seed Laboratory, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Seedling bioassay

Radicle length (cm) of each seedling was measured from the hypocotyl end to the root tip of each seedling by using a ruler while radicle diameter (mm) was measured at the midpoint of the radicle using a digital venire caliper (CD6'CSX, Mitutoyo, USA). Hypocotyl length was measured (cm) with a ruler from the tip of the shoot until just before the radicle part.

For fresh weight measurement, each of the seedlings was weighed by using a digital weighing scale (Model AJ100, Mettler, Switzerland) rapidly after being taken out from the petri dish in order to avoid water loss from the seedlings. Then, all the seedlings from each sample were put into an individual paper envelope and dried in an oven (Memmert, Germany) for 72 hours at 50 °C. Dry weight was measured using the weighing scale above.

Seed germination

For leaf lettuce, cucumber and green amaranth, the total seed germination rateswere measured three days after the seeds had germinated. As for tomato, the total seed germination rate was measured after 4 days of germination since tomato seeds needed a longer time to germinate compared with the other vegetables used. Percentage seed germination was calculated using the following formula:

$$Seed\ germination\ (\%) = \frac{Number\ of\ germinated\ seeds\ \times 100}{Number\ of\ seeds\ used\ in\ bioassay}$$

Mean germination time

The mean germination time was calculated by using the folowing formula (Czabator, 1962):

$$Mean\ germination\ time = \frac{(N1 \times 1) + (N2 \times 2) + (N3 \times 3) + (Nn \times n)}{N1 + N2 + N3}$$

N1 = Seed germinated on day 1

N2 = Seed germinated on day 2

N3 = Seed germinated on day 3

N_n= Seed germinated on day n

Experimental design and statistical analysis

The experiment was conducted using the randomized complete block design with four replications. Each treatment consisted of four different oil palm wastes, OPT, EFB, PPMF, and OPF, and with ten sub-samples of seedling for each vegetable, leaf lettuce, tomato, cucumber and green amaranth. Data were analysed using the analysis of variance and significant treatment means were separated by least significant difference at P≤0.05 (SAS, version 9.4).

Results and Discussion

Radicle length

Results from the seedling bioassay study showed that different oil palm waste aqueous extracts significantly influenced elongation of leaf lettuceradicle (Figure 1a). The maximum inhibition of radicle growth was observed in OPF aqueous extract followed by PPMF and EFB aqueous extracts. Radicle elongation was significantly reduced by 62% and 56% of control for OPF and PPMF aqueous extracts, respectively, whereas for EFB, the radicle elongation was reduced by 24 of control. However, the OPT extract stimulated growth of lettuce radicle by 12.9% of control.

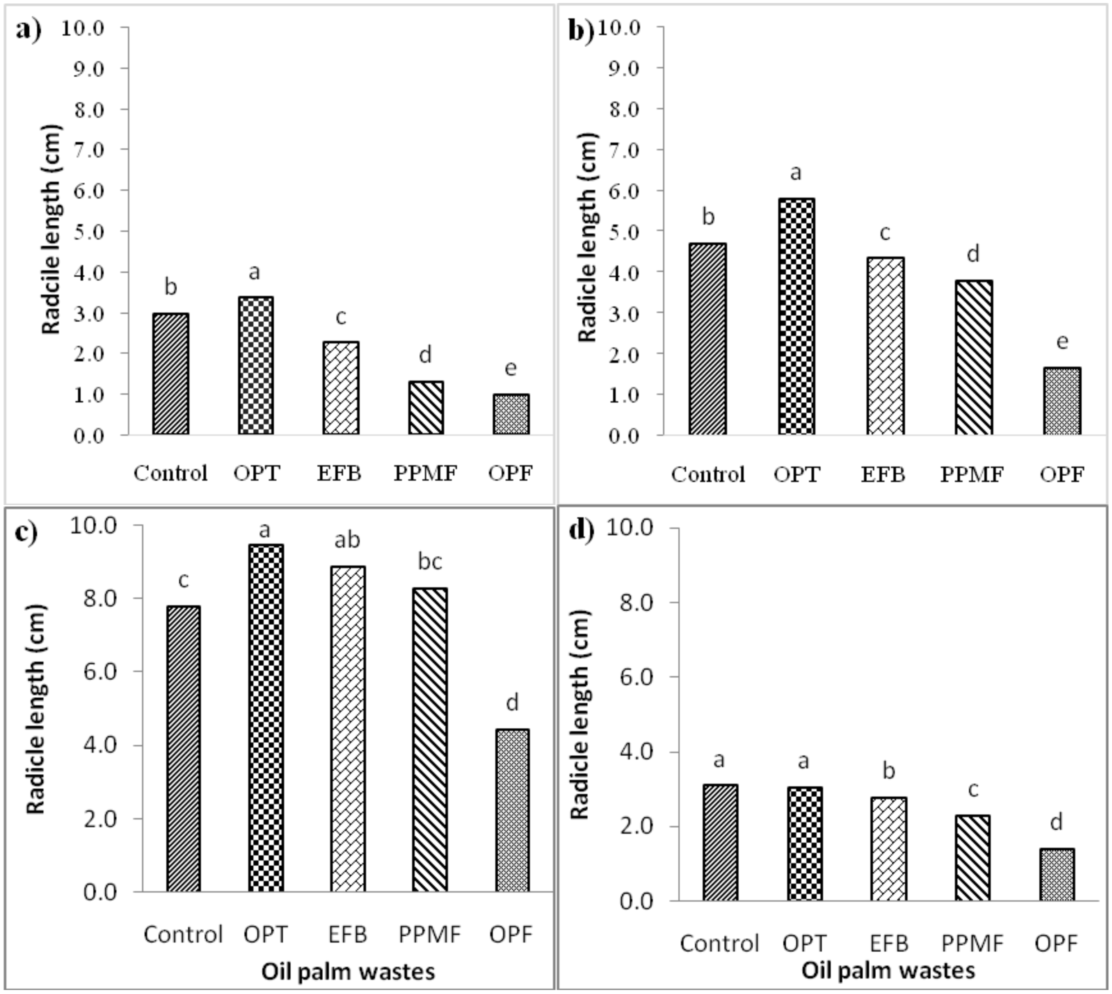


Figure 1.Radicle length of a) lettuce (Lactuca sativa), b) tomato (Solanum lycopersicum), c) cucumber (Cucumis sativus) and d) green amaranth (Amaranthus viridis) treated with waste aqueous extract of oil palm trunk (OPT), empty fruit bunch (EFB), palm pressed mesocarp fiber (PPMF), and oil palm frond (OPF). Means with different letters above each bar indicate significant differences at $P \leq 0.05$ using LSD test.

A similar trend was observed when tomato seedlings were treated with different type of oil palm waste aqueous extracts (Figure 1b). The highest inhibition for radicle growth occurred when seeds were treated with OPF aqueous extract followed by PPMF and EFB aqueous extracts. OPF extract reduces the radicle length by 65% of control. PPMF and EFB extracts produced seedlings with 20% and 8% shorter radicle length, respectively, compared to control. OPT extract produced 23% longer radicle compared to control.

ISSN: 2456-8643

The radicle length of cucumber were significantly stimulated by the different type of applications of oil palm wastes aqueous extracts (Figure 1c). Applications of OPT, EFB and PPMF aqueous extracts sgnificantly increased radicle length of cucumber seedlings compared to control. The application of oil palm waste extracts produced more than 11% longer radicle length compared to control. However, the OPF extract inhibited radicle elongation resulting in 53% shorter radicle compared to control.

Applications of oil palm waste aqueous extracts on green amaranthseedlings significantly affected radicle lengths (Figure 1d). Radicle lengths of green amaranth treated with EFB, PPMF and OPF extracts were inhibited and these oil palm wastes produced shorter radicle length compared to control. The most phytotoxic waste extract was exhibited by the OPF extract. The radicle length of green amaranth seedling was 53% shorter when treated with OPF extractwhile PPMF extract reduced radicle length by 24% and EFB by 11% when compared to control. OPT waste showed no inhibition on radicle length of green amaranth seedlings.

Root or radicle elongation has always been used as an indicator of the presence of phytotoxic compounds since this plant part is very sensitive and easily respond to the presence of toxicity (Wang, 1985), and the radicle is the first plant part that emerge to have contact with the compound. Besides that, the radicle is responsible for absorption and accumulation of any compound which make the radicle very sensitive to the presence of toxic compounds compared with other plant parts (Öncel et al., 2000). Based on the results, the reduction in radicle length by EFB, PPMF and OPF extracts showed the influence of these three wastes on the radicle growth of leaf lettuce, tomato and green amaranth. Only OPF extract inhibited growth of cucumber seedlings while the other wastes caused stimulation of radicle growth of the seedlings.

Thus, the OPF contained a strong phytotoxic compound compared with the other oil palm wastes. The differences in the trend of radicle growth among four different species of seedlings could be due to the size of seedlings. The small seedlings, like green amaranth, tomato and lettuce, were inhibited when applied with EFB, PPMF and OPF extracts (Cheung et al., 1989). This was because small seeds and seedlings usually rely on the radicles to get food and contain small amounts of food reserve for survival (Cheung et al., 1989). However, the big vegetable seeds and seedlings, such as cucumber, contained a high reserve of food causing the radicle growth to be more tolerant towards application of oil palm wastes aqueous extracts except for the OPF (Tiquia et al., 1996). Also, the ability of phytotoxic compound to inhibit the growth of seedlings depended on the species of the seedlings since some of the specific phytotoxic compound might only just affect the growth of a particular species and not all plant species (Chick and Kielbaso, 1998).

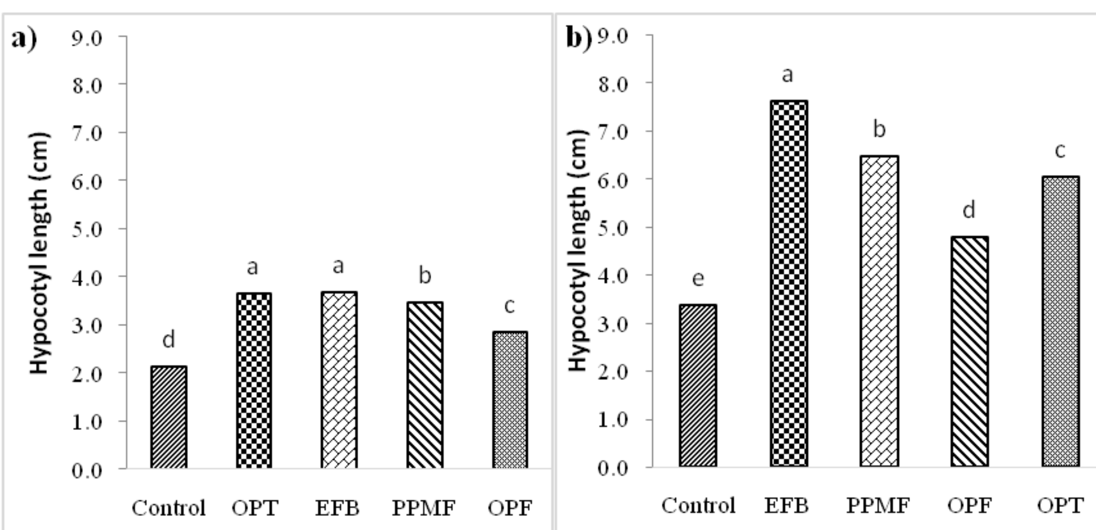
ISSN: 2456-8643

Hypocotyl length

There were significant differences in hypocotyl lengths between the control and oil palm waste treated lettuce (Figure 2a). However, there were no inhibitions on radicle length of the seedlings when treated with different aqueous extracts of the oil palm wastes compared to control. The applications of aqueous extracts stimulated the elongation of hypocotyl significantly compared to control. The hypocotyl was elongated from 34% to 73% compared to control. However, the hypocotyl length for lettuce seedlings treated with OPF aqueous extract showed the shortest length among the oil palm waste extracts.

The hypocotyl lengths increased significantly with the application of different oil palm waste aqueous extracts on tomato seedlings (Figure 2b). There were no inhibitions in hypocotyl growth with application of oil palm wastes aqueous extracts. The application of EFB extract on tomato seedlings gave the longest hypocotyl length (126%) followed byextracts of PPMF (92%) and OPT (80%) compared with control. The shortest increment of hypocotyl lengths was measured on seedlings treated with OPF extract, with 42% increase compared with control.

Figure 2c showed that hypocotyl length of seedlings treated with OPT, EFB and PPMF extracts showed no inhibition. Furthermore, hypocotyl length of cucumber seedlings treated with PPMF were stimulated by 23% compared to control. However, only the OPF extract produced significantly shorter hypocotyl length of cucumber seedlings, by 47% compared to the control.



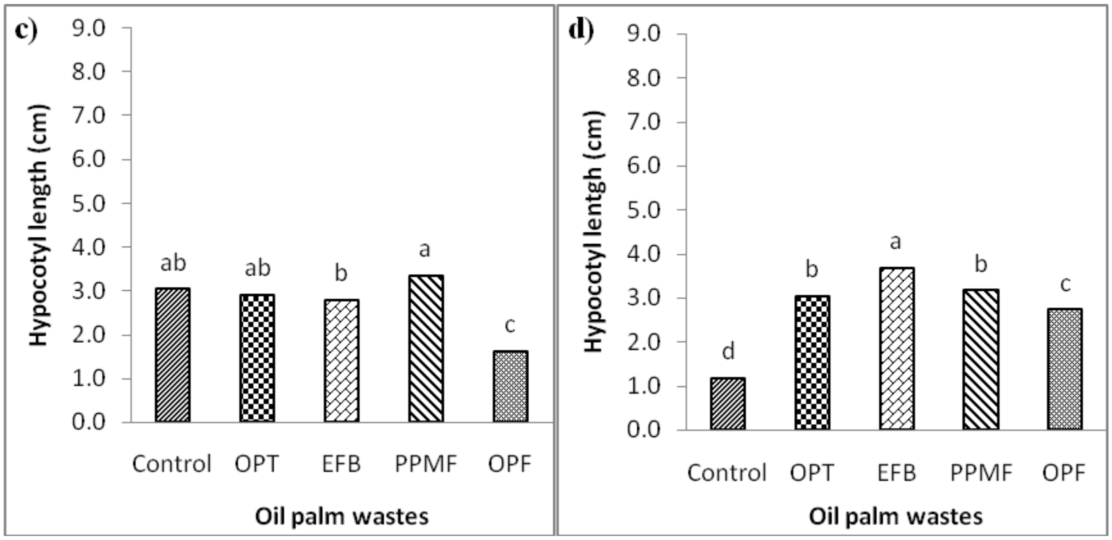


Figure 2. Hypocotyl length of a) lettuce (Lactuca sativa), b) tomato (Solanum lycopersicum), c)cucumber(Cucumis sativus) and d) green amaranth (Amaranthus viridis) treated with waste aqueous extract of oil palm trunk (OPT), empty fruit bunch (EFB), palm pressed mesocarp fiber (PPMF), and oil palm frond (OPF). Means with different letters above each bar indicate significant differences at P ≤ 0.05 using LSD test.

Hypocotyl length of 5-day old green amaranth seedlings were significantly longer when seedlings were treated with oil palm waste extracts (Figure 2d). Seedling hypocotyl length treated with different type of oil palm wastes were increased significantly from 102% to 187% longer hypocotyl length than control. The longest hypocotyl length was measured when green amaranth was treated with EFB aqueous extract and the shortest was by the OPF aqueous extract.

Based on the overall results of hypocotyl length, only the cucumber seedlings showed inhibition when treated with the OPF extract. Whereas, other oil palm waste extracts showed no inhibition on hypocotyl length, including the OPF extract when applied on seeds of green amaranth, tomato and leaf lettuce. A similar result also was found when soybean and chive seedlings were treated with aqueous extract of ginger, whereby there was no inhibition on the hypocotyl development, while only the radicle was affected by application of the ginger extract (Han et al.,2008). The hypocotyl lengths were not affected by the presence of phytotoxic compounds since the seedlings were still dependent on food reserve present in the seeds. However, the radicle was easily affected by phytotoxic compounds because this plant part had direct contact with the phytotoxic compounds present in the media and was responsible for absorption of the particular

compound (Turk and Tawaha, 2002). This resulted in the hypocotyls not being easily affected by external contamination during early stage of seedling growth compared to radicles (Migliore et al., 2003).

Radicle diameter

Radicle diameter of lettuce from the control was significantly higher than those of lettuce seedlings treated with different types of oil palm wastes (Figure 3a). The seedlings treated with oil palm waste extracts produced smaller radicle diameter, by 10% to 11%, compared to control. However, among the oil palm waste extract treatments, there were significant differences in radicle diameter between OPT extract treatment compared to those treated with EFB, PPMF and OPF extracts.

There were significant differences between radicle diameter for tomato seedlings treated with OPT and PPMF extracts compared to the control (Figure 3b). The radicle diameter of tomato seedlings treated with OPF and PPMF extracts was increased by 9% and 4% of control. However, there were no significant differences in tomato seedlings treated with EFB and OPF extracts, but radicle diameter of tomato seedlings treated with OPT was significantly bigger compared with other oil palm wastes.

Applications of oil palm waste extracts significantly affected the radicle length of cucumber seedlings (Figure 3c). In general, all oil palm waste extracts decreased the radicle length of cucumber seedlings compared to control although OPT extract gave slightly smaller radicle diameter of cucumber seedling by 6% of control. Meanwhile, EFB, OPF and PPMF extracts reduced the radicle diameters by 11 to 12% compared to control. However, there were no significant differences in radicle diameters with treatments of OPT and PPMF extracts.

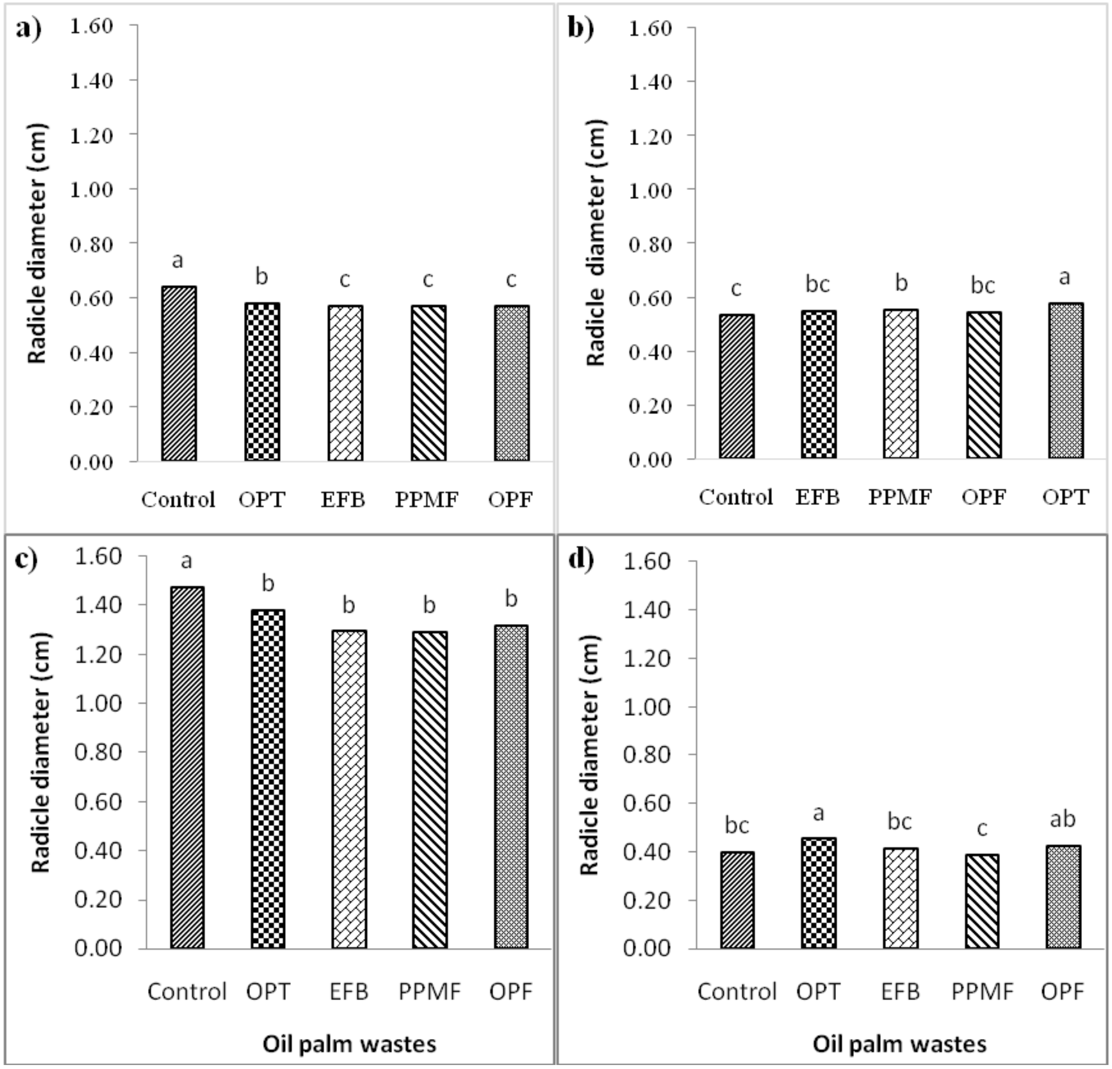


Figure 3. Radicle diameter of a) lettuce (Lactuca sativa), b) tomato (Solanum lycopersicum, c)cucumber(Cucumis sativus) and d) green amaranth (Amaranthus viridis) treated with waste aqueous extract of oil palm trunk (OPT), empty fruit bunch (EFB), palm pressed mesocarp fiber (PPMF), and oil palm frond (OPF). Means with different letters above each bar indicate significant differences at $P \leq 0.05$ using LSD test.

There were different effects of the oil palm waste extracts on the radicle diameter of green amaranth seedlings treated with OPT and OPF extracts compared to control (Figure 3d). However, there were no significant differences in seedlings radicle diameter when the seedlings were treated with PPMF and EFB. The radicle diameters of seedlings treated with OPT and OPF extracts were significantly higher compared to control.

Based on overall results of radicle lengths treated with different types of oil palm wastes, the response of radicle length to applications of oil palm waste extracts varied among species of seedlings. Lettuce and cucumber seedlings showed reduction in radicle length when treated with OPT, EFB, PPMF and OPF extracts compared to control. However, for tomato seedlings, only OPF treated seedlings showed reduction in radicle lengths, while OPT, EFB and PPMF showed no differences in radicle lengths compared with control. Several studies showed that the presence of phytotoxic compunds could affect the size of the root or radicle part of the plant. Vaughan and Ord (1990) reported that phenolic acids, such as ferulic, vanillic, *p*-hydroxybenzoic, syringic, and caffeic acid, which are phytotoxic compounds, inhibited pea root growth. These phenolic acids also affected root morphology in terms of growth of the main root and the number and size of lateral roots.

Studies by Batish et al. (2006) on the phytotoxicity of volatile essential oilsreported that essential oils and their constituents inhibited cell division in growing root tips and interfere with DNA synthesis in growing meristems. On the other hand, green amaranth seedlings responded differently compared with other seedlings. The radicle diameter of green amaranth seedlings was enhanced when treated with OPT and OPF extracts. A similar increase in radicle diameter was found for alfalfa seedlings when treated with coumarin and alfalfa aqueous extract (Chon et al., 2002). The increase in the radicle diameter could be because of the increase in the vascular cylinder and cortex cell layers inside the root which caused radicle to become thick (Chon et al., 2002).

Fresh weight

Results from the bioassay studies showed that there were significant differences between fresh weights of lettuce seedlings treated with EFB, OPT, PPMF and OPF extracts (Figure 4a). Fresh weights of lettuce were increased significantly when treated with EFB, OPT and PPMF extracts by 44%, 29% and 17% of control, respectively. Meanwhile, OPF extract reduced fresh weight of seedlings significantly by 9% of control.

There were different effects of the OPT, EFB and PPMF extracts on fresh weights of tomato seedlings (Figure 4b). Fresh weights of tomato seedlings treated with OPT, EFB and PPMF extracts were significantly higher by 30%, 37% and 13%, respectively, compared to control. Whereas, fresh weight of tomato seedlings treated with OPF extract was not significantly reduced.

There were different effects of the PPMF and OPF extracts on the fresh weights of cucumber seedlings (Figure 4c). The seedling fresh weight of PPMF extract treatment was significantly higher compared to the control and those treated with EFB and OPF extracts. The fresh weight of PPMF treated seedlings resulted in 11% higher fresh weight compared to control. However, application of the OPF extract on cucumber seedlings produced significantly lower fresh weight compared to other treatments including the control. Fresh weight of cucumber seedling was reduced by 31% compared to control.

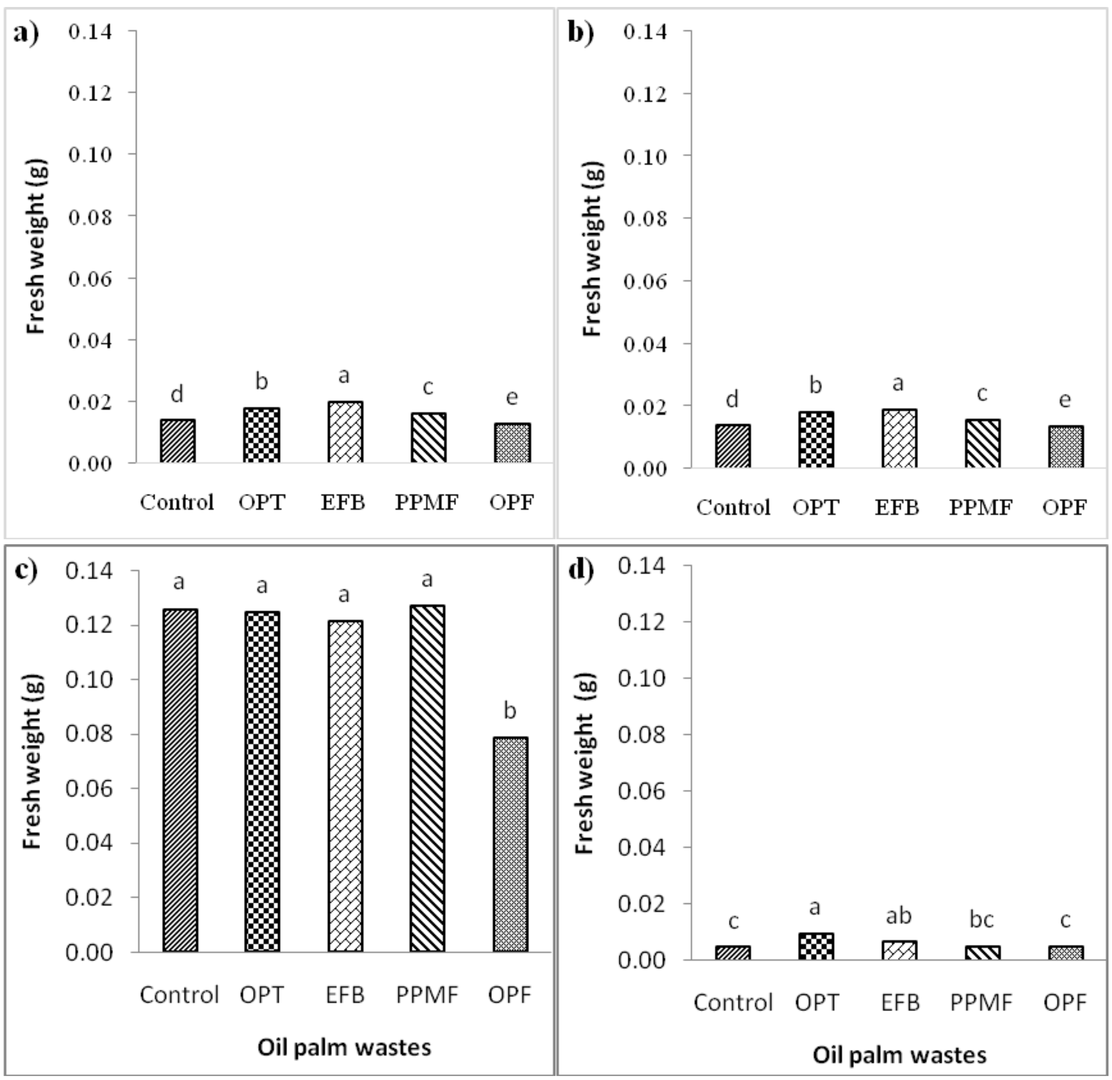


Figure 4. Fresh weight of a) lettuce (Lactuca sativa), b)tomato (Solanum lycopersicum), c) cucumber(Cucumis sativus) and d) green amaranth (Amaranthus viridis) treated with waste

ISSN: 2456-8643

aqueous extract of oil palm trunk (OPT), empty fruit bunch (EFB), palm pressed mesocarp fiber (PPMF), and oil palm frond (OPF). Means with different letters above each bar indicate significant differences at P ≤ 0.05 using LSD test.

There were significant differences between fresh weights of green amaranth seedlings when treated with OPT and EFB extracts compared to control (Figure 4d). Fresh weights of seedlings that weretreated with OPT and EFB extracts were significantly higher than control by 35% and 44%. However, only EFB extract produced higher fresh weight of green amaranth seedlings compared to PPMF and OPF extracts. For PPMF and OPF extracts, there were no significant effect on fresh weight of green amaranth compared to control.

The overall results showed that the effect of oil palm waste extracts application varied among types of oil palm wastes and seedlings. Almost all extracts produced higher fresh weights of seedlings compared to control except the OPF extract. OPF extract showed decreased fresh weight of cucumber seedling compared to control and other treatments. The decrease in fresh weight of cucumber seedling treated with OPF extract indicated that OPF hadphytotoxicity effect on cucumber seedling. Previous studies showed that the phytotoxic compound could reduce the total biomass of plants. Lee et al. (2006) stated that organic acids that contain phenolic acids in nutrient solution reduced growth of leaf area, shoot fresh and dry weights, and root fresh weight of lettuce seedlings. According to Premuzic et al., (2007) the decrease in fresh weight is because of phytotoxic compound affecting the shoot growth causing lack of vigour and foliage chlorosis on vegetable seedlings. Ambrosio de Castro (2006) reported that higher ammonia concentrations caused toxic effect to *Brasicca oleracea* by reducing biomass production of roots. OPT and EFB extracts produced higher total biomass for lettuce, tomato and green amaranth seedlings and there were no differences in fresh weights of cucumber seedlings compared to control. Meanwhile, PPMF extract also showed no decreased in fresh weight of seedlings compared to control. According to Han et al.(2008), radicle length was found to be the more sensitive plant part when expose to the phytotoxic compounds compared to shoot length, germination test and total biomass of seedlings.

Seedling dry weight

There were no significant differences between the means of the dry weights for both the lettuce and tomato seedlings treated with oil palm waste extracts compared to the control (

Table 1).

ISSN: 2456-8643

Table 1. Dry weights of leaflettuce (Lactuca sativa)and tomato (Solanum lycopersicum), treated with waste aqueous extracts of oil palm trunk (OPT), empty fruit bunch (EFB), palm pressed mesocarp fiber (PPMF), and oil palm frond (OPF).

Oil palm wastes	Seedling dry weight (mg)	
	Leaf Lettuce	Tomato
Control (distilled water)	10.1 a	16.5 a
OPT	10.1 a	16.6 a
EFB	10.4 a	16.8 a
PPMP	10.4 a	15.7 a
OPF	10.6 a	16.4 a

Mean values (n = 4) within the same column followed by the same letter are not significantly different at $P \geq 0.05$ by LSD test.

The dry weight of the cucumber seedlings treated with the OPF extract was significantly lower compared to the control seedlings and those treated with OPT, EFB and PPMF extracts (Figure 5a). The OPF extract treatment resulted in 15% lower seedling dry weight compared to the control.

There were significant differences between the dry weight for green amaranth seedlings treated with PPMF extract compared to the EFB extract (Figure 5b). The application of EFB extract on green amaranth seedlings produced 33% higher dry weight compared to dry weight of seedlings treated with the PPMF extract. However, there were no significant differences in dry weights of green amaranth seedlings treated with OPT, EFB, PPMF and OPF extracts compared to the control.

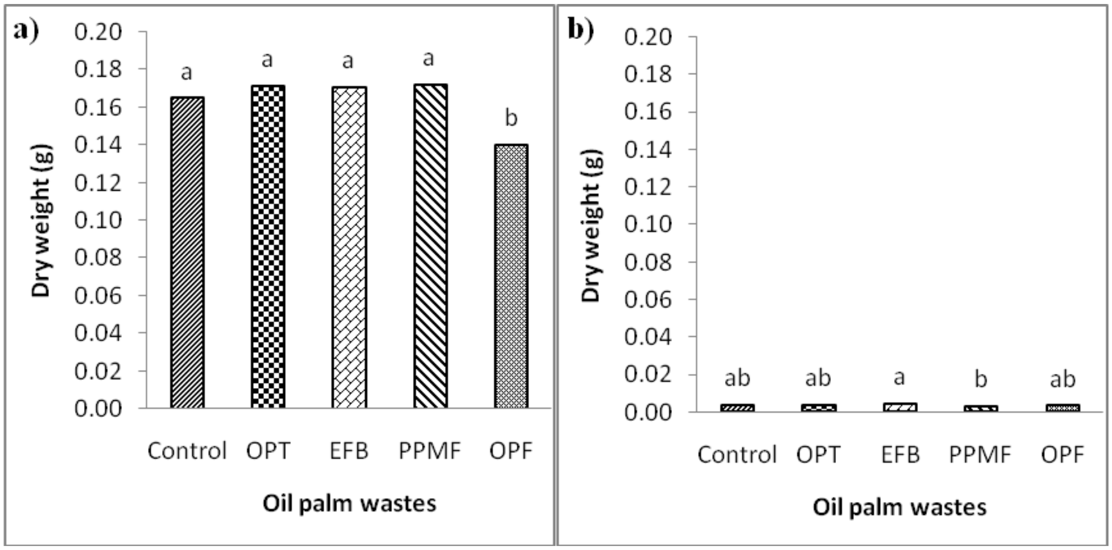


Figure 5. Dry weight of a) cucumber (Cucumis sativus) and b) green amaranth (Amaranthus viridis) seedlings treated with waste aqueous extracts of oil palm trunk (OPT), empty fruit bunch (EFB), palm pressed mesocarp fiber (PPMF), and oil palm frond (OPF). Means with different letters above each bar indicate significant differences at P ≤ 0.05 using LSD test.

The overall results showed that only the OPF extract reduced the dry weight of cucumber seedlings significantly compared to the control. For the other type of seedlings, the application of oil palm wastes extracts did not affect the dry matter weight of seedlings compared to the control. Dry matter weight refers to entire organic dry matter that is produced from the essential activities of photosynthesis and protein metabolism (Fageria, 2007). A study on the effect of phytotoxicity of *Parthenium hysterophorus* leaf extract showed some physiological changes, such as damage of cellular membrane, loss of dehydrogenase activity in roots and decreased water content in leaves (Batish et al., 2007). Such interruption of physiological process in a crop also causes reduction on the *Brassica* sp. seedlings fresh and dry weights significantly (Singh et al., 2005). This occurred due to the release of some growth-retarding substances from the plant residues into the soil, which accumulate in bioactive concentrations and adversely affect plant growth. Chon and Kim (2005) reported that a coumarin compound, which is one of the phytotoxic compounds, inhibited dry matter production, leaf area expansion and photosynthesis in tobacco (*Nicotiana tabacum L.*), sunflower (*Helianthus annuus* L.) and red root amaranth (*Amaranthus retroflexus)*.

Total germination rate

The results from the seed bioassay studies showed that the different oil palm wastes aqueous extracts did not significantly influenced the total seed germination rates of leaf lettuce, tomato, cucumber and green amaranth (Table 2).

Table 2. Total seed germination rate of lettuce (Lactuca sativa),tomato (Solanum lycopersicum), cucumber (Cucumis sativus) and green amaranth (Amarantus viridis) treated with waste aqueous extracts of oil palm trunk (OPT), empty fruit bunch (EFB), palm pressed mesocarp fiber (PPMF), and oil palm frond OPF.

Oil palm wastes	Total seed germination rate (%)			
	Leaf Lettuce	Tomato	Cucumber	Green Amaranth
Control	92.5 a	100.0 a	100.0 a	85.0 a
OPT	87.5 a	100.0 a	100.0 a	80.0 a
EFB	85.0 a	100.0 a	97.5 a	75.0 a
PPMF	92.5 a	97.5a	97.5 a	82.5 a
OPF	85.0 a	95.0a	97.5 a	87.5 a

Mean values (n = 4) in the same column followed by the same letter are not significantly different at $P \geq 0.05$ by LSD test.

In general, seed germination for lettuce, tomato, cucumber and green amaranth was not affected by oil palm waste applications. Seed germination and seedling growth are widely used as methods to determine the presence of phytotoxic compounds in plant wastes (Kapanen and Itavaara, 2001). Araújo and Monteiro (2005) stated that seed germination was less sensitive compared to plant growth for the purpose of determining the presence of phytotoxicity. This is because of the seed reserve that contains a huge bunch of nutrients (carbohydrate, minerals, fibre, enzymes, etc)are made available for seed germination during the early stage of growth. Besides, Kapustka (1997) stated that the seed germination bioassay relatively is tolerant to many toxic compounds which may harm the plant growth. Moreover, the tolerance of seeds toward a phytotoxic compound could be because many of the chemical compounds are not absorbed by the seeds and the seeds already contain nutrients which are sufficient for their growth during the germination period, thus restricting them from relying on any nutrients or chemical compounds from the surrounding the environment (Kapustka, 1997). However, at a higher concentration, the phytotoxic compounds have the probability to affect the germination rate of the seeds (Chung and Miller, 1995). This indicated that seed germination has a negative correlation with the concentration of phytotoxic compound (Mahmoodzadeh and Mahmoodzadeh, 2013).

Mean germination time

According to the results, the mean germination time in the control treatment was the shortest compared to lettuce seeds treated with the other type oil palm wastes (Figure 6). The seeds treated with OPF, PPMF and OPT needed more time to germinatefollowed by those treated with EFB, compared to the control.

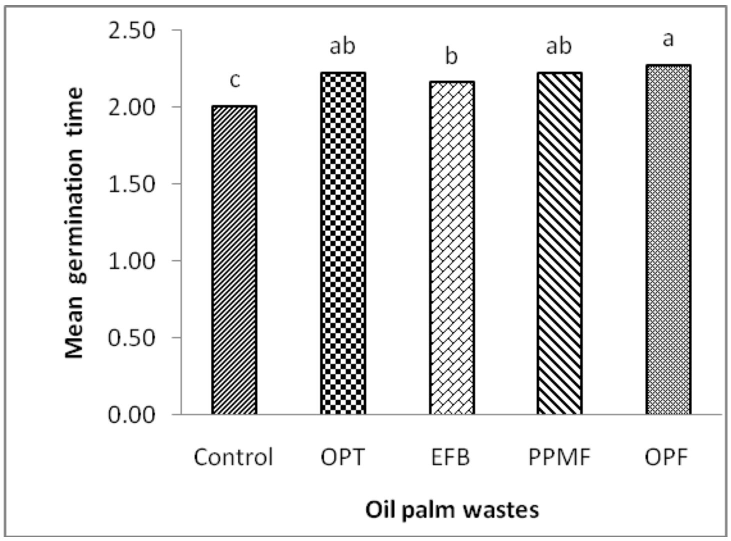


Figure 6. Mean germination time of lettuce (Lactuca sativa, treated with waste aqueous extracts of oil palm trunk (OPT), empty fruit bunch (EFB), palm pressed mesocarp fiber (PPMF), and oil palm frond (OPF). Means with different letters above each bar indicate significant differences at P ≤ 0.05 using LSD test.

There were no significant differences on the mean germination time for the tomato, cucumber and green amaranth seeds to germinate when the seeds were treated with OPT, EFB, PPMF and OPF extracts compared to the control (Table 3).

Vol. 2, No. 04; 2017

ISSN: 2456-8643

Table 3. Mean germination time of tomato (Solanum lycopersicum), cucumber (Cucumis sativus) and green amaranth (Amarantus viridis) to germinate when treated with waste aqueous extracts of oil palm trunk (OPT), empty fruit bunch (EFB), palm pressed mesocarp fiber (PPMF), and oil palm frond OPF.

Oil palm wastes	Mean germination time		
	Tomato	Cucumber	Green Amaranth
Control	3.21 a	2.01 a	3.00 a
OPT	3.29 a	2.02 a	3.02 a
EFB	3.30 a	2.03 a	3.04 a
PPMF	3.20 a	2.04 a	3.05 a
OPF	3.29 a	2.05 a	3.00 a

Mean values (n = 4) in the same column followed by the same letter are not significantly different at $P \geq 0.05$ by LSD test.

Application of OPF extracts on lettuce delayed seed germination significantly compared to control. However, the tomato, cucumber and green amaranth seedlings showed no effect with the application of oil palm waste aqueous extracts on average days for seeds to germinate. Base on the overall results, the sensitivity of this parameter towards the application of oil palm wastes was greater than total seed germination. According to Alencar et al. (2015), speed of seed to germinate is a more reliable measure of the presence of phytotoxic compounds compared to total seed germination because the phytotoxic compounds may not inhibit the seed germination but may delay seed germination process. Also, delays in germination will affect the growth of seedlings under natural a environment (Chaves et al., 2001) because this would minimize the chance of seedlings to compete with other seedlings for resources, thus reducing the chances for seedlings to grow well (Gao et al., 2009). However, Haugland and Brandsaeter (1996) stated thatradicle growth is the most sensitive part to detect the presence of phytotoxic compounds in plant, which determines the compound's phytotoxicity.

Conclusions

Based on seedling bioassay and seed germination of different seedlings, the OPF aqueous extract was found to be the most inhibitory oil palm waste extract compared with other treatments. OPF inhibited seedling growth by reducing the radicle length for all seedlings, hypocotyl length of cucumber, fresh weight of cucumber, dry weight of lettuce, tomato and cucumber seedling and mean germination time. While PPMF aqueous extract reduced the radicle length of lettuce, tomato and green amaranth seedling with a higher degree of inhibition comparing with EFB. Radicle elongation is the main criteria to detect the present of phytotoxic compound with support from other parameters.

REFERENCES

Abdullah, N. and F. Sulaiman. 2013. "The Oil Palm Wastes in Malaysia." In Biomass Now - Sustainable Growth and Use, edited by D. M. Miodrag, 75–99. INTECH Open Access Publisher.

Ahmad, F. (2001, September). Sustainable agriculture system in Malaysia. In Regional Workshop on Integrated Plant Nutrition System (IPNS), Development in Rural Poverty Alleviation, United Nations Conference Complex, Bangkok, Thailand (pp. 18-20).

Alencar, S. R., Silva, M. A. P., Matos, M. F., Santos, M. A. F., Generino, M. E. M., Torquato, I. H. S., and Crispim, M. K. M. (2015). Biological Activity of Bambusa vulgaris Schrad. ex J.C. Wendl.(Poaceae). Journal of Agricultural Science, 7(6), 150-159.

Ali, M. F. and Abbas, S. 2006. A Review of Methods for the Demetallization of Residual Fuel Oils.Fuel Processing Technology 87 (7): 573–84.

Ambrosio de Castro, A. H. 2006.Atmospheric NH3 deposition, S and N metabolism in curly kale, Doctor of Philosophy, University of Groningen, Netherlands.

Araújo, A. S. F. and Monteiro, R. T. R. (2005). Plant Bioassays to Assess Toxicity of Textile Sludge Compost. Scientia Agricola, 62(3), 286-290.

Batish, D. R., Singh, H. P., Setia, N., Kaur, S.and Kohli, R. K. (2006). Chemical Composition and Phytotoxicity of Volatile Essential Oil from Intact and Fallen Leaves of Eucalyptus citriodora. Zeitschrift für Naturforschung C, 61(7-8), 465-471.

Batish, D. R., Kaur, M., Singh, H. P., and Kohli, R. K. (2007). Phytotoxicity of A Medicinal Plant, Anisomeles indica, against Phalaris minor and its Potential Use as Natural Herbicide in Wheat Fields.Crop Protection,26(7), 948-952.

Bhowmik, P. C. (2003). Challenges and Opportunities in Implementing Allelopathy for Natural Weed Management. Crop Protection, 22(4), 661-671.

Bogatek, R., Gniazdowska, A., Zakrzewska, W., Oracz, K., and Gawronski, S. W. (2006).Allelopathic Effects of Sunflower Extracts on Mustard Seed Germination and Seedling Growth. Biologia Plantarum, 50(1), 156-158.

Chaves, N., Sosa, T., and Escudero, J. C. (2001). Plant growth inhibiting flavonoids in exudate of Cistus ladanifer and in associated soils. Journal of chemical ecology, 27(3), 623-631.

Cheung, Y. H., Wong, M. H. and Tam, N. F. Y. (1989). Root and Shoot Elongation as An Assessment of Heavy Metal Toxicity and ‘Zn Equivalent Value’of Edible Crops.In Environmental Bioassay Techniques and their Application (pp. 377-383). Springer, Netherlands.

Chick, T. A. and Kielbaso, J. J. (1998). Allelopathy as An Inhibition Factor in Ornamental Tree Growth: Implications from The Literature. Journal of Arboriculture, 24(5), 274-279.

Chon, S. U., Choi, S. K., Jung, S., Jang, H. G., Pyo, B. S. and Kim, S. M. (2002). Effects of Alfalfa Leaf Extracts and Phenolic Allelochemicals on Early Seedling Growth and Root Morphology of Alfalfa and Barnyard Grass. Crop protection, 21(10), 1077-1082.

Chon, S. U. and Kim, D. K. (2005). Allelopathic Potential of Xanthium occidentaleExtracts and Residues.Korean Journal of Weed Science, 30(2), 349-358.

Chung, I. M. and Miller, D. A. (1995). Natural Herbicide Potential of Alfalfa Residue on Selected Weed Species.Agronomy Journal,87(5), 920-925.

Czabator, F. J. (1962). Germination Value: An Index Combining Speed and Completeness of Pine Seed Germination. Forest Science, 8(4), 386-396.

Dilipkumar, M., Mazira, C. M. and Chuah,T. S. 2015. Phytotoxicity of Different Organic Mulches on Emergence and Seedling Growth of Goosegrass (Eleusine indica). Journal Tropical Agriculture and Food Science,43(2), 145–53.

Fageria, N. K. (2007). Yield Physiology of Rice.Journal of Plant Nutrition,30(6), 843-879.

Gao, X., Li, M. E. I., Gao, Z., Li, C. and Sun, Z. (2009). Allelopathic Effects of Hemistepta lyrataon The Germination and Growth of Wheat, Sorghum, Cucumber, Rape, and Radish Seeds.Weed Biology and Management, 9(3), 243-249.

Han, C. M., Pan, K. W., Wu, N., Wang, J. C., and Li, W. (2008). Allelopathic effect of ginger on seed germination and seedling growth of soybean and chive. Scientia horticulturae, 116(3), 330-336.

Haugland, E. and Brandsaeter, L. O. (1996). Experiments on Bioassay Sensitivity in The Study of Allelopathy. Journal of Chemical Ecology,22(10), 1845-1859.

Kapanen, A. and Itävaara, M. (2001). Ecotoxicity Tests for Compost Applications. Ecotoxicology and environmental safety, 49(1), 1-16.

Kapustka, L. A. (1997). Selection of Phytotoxicity Tests for Use in Ecological Risk Assessments. In: Wang, W., Gorsuch, J.W. and Hughes, D. Plants for Environmental Studies. New York: CRC Press, pp.516-548.

Kerdsuwan, S. and Laohalidanond, K. (2011).Renewable Energy from Palm Oil Empty Fruit Bunch. INTECH Open Access Publisher.

Lee, J. G., Lee, B. Y. and Lee, H. J. (2006). Accumulation of Phytotoxic Organic Acids in Reused Nutrient Solution During Hydroponic Cultivation Of Lettuce (Lactuca sativa L.).Scientia horticulturae,110(2), 119-128.

Migliore, L., Cozzolino, S. and Fiori, M. (2003). Phytotoxicity to and Uptake of Enrofloxacin in Crop Plants. Chemosphere, 52(7), 1233-1244.

Mahmoodzadeh, H. and Mahmoodzadeh, M. (2013). Allelopathic Potential of Soybean (Glycine max L.) on the Germination and Root Growth of Weed Species. Life Science Journal,10, 63-69.

Mohammad, A., Yazid, M., Abdul Razak, M., Ismail, M. M. and Islam, M. A. (2012, 5-6 July 2012). Oil Palm Fronds (OPF) as Potential Affordable Source of Feeds for Ruminants Small Holder Farms. Paper presented at the 2nd International Seminar on Animal Industry. Jakarta,

MPOB. 2014. Oil Palm Planted Area by State as at December 2014 [Electronic version]. http://bepi.mpob.gov.my/images/area/2014/Areasummary.pdf.

Öncel, I., Keleş, Y. and Üstün, A. S. (2000). Interactive Effects of Temperature and Heavy Metal Stress on the Growth and Some Biochemical Compounds in Wheat Seedlings. Environmental Pollution,107(3), 315-320.

Premuzic, Z., Palmucci, H. E., Tamborenea, J. and Nakama, M. (2007). Chlorine Disinfection: Effects on Hydroponics Lettuce.The Society for Advancement of Horticulture,9(1), 62 -65.

Radziah, O., Rahmani, M. and Hashim, A. 1997. Phytotoxicity of Phenolic Acids Extracted from Palm Oil Dry Solids.Pertanika Journal of Tropical Agricultural Science, 2(2/3): 91–99.

Singh, H. P., Batish, D. R., Pandher, J. K. and Kohli, R. K. (2005). Phytotoxic Effects of Parthenium hysterophorus Residues on Three Brassica species.Weed Biology and Management,5(3), 105-109.

Suhaimi, M. and Ong, H. K. (2001). Composting Empty Fruit Bunches of Oil Palm. Extension Bulletin-Food and Fertilizer Technology Center, (505), 1-8.

Tiquia, S., Tam, N. and Hodgkiss, I. (1996). Microbial Activities during Composting of Spent Pig-manure Sawdust Litter at Different Moisture Contents. Bioresource Technology, 55(3), 201-206.

Turk, M. A. and Tawaha, A. M. (2003). Allelopathic Effect of Black Mustard (Brassica nigra L.) on Germination and Growth of Wild Oat (Avena fatua L.).Crop Protection,22(4), 673-677.

Vaughan, D. and Ord, B. (1990). Influence of Phenolic Acids on Morphological Changes in Roots of Pisum sativum. Journal of the Science of Food and Agriculture, 52(3), 289-299.

Wang, W. (1985). The use of Plant Seeds in Toxicity Tests of Phenolic Compounds. Environment International, 11(1), 49-55.

Wang, X., Dong, Y., Han, S. and Wang, L. (2000). Structure–phytotoxicity Relationship: Comparative Inhibition of Selected Nitrogen-containing Aromatics to Root Elongation of Cucumis sativus.Bulletin of Environmental Contamination and Toxicology, 65(4), 435-442.