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# COMBINATION RATIO AFFECTS SYNERGISTIC ACTIVITY OF OIL PALM FROND RESIDUE AND S-METOLACHLOR ON GOOSEGRASS (*ELEUSINE INDICA*)

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**Abstract**

Goosegrass (*Eleusine indica*), a troublesome weed, has been documented to evolve resistance to various groups of herbicides. Recent field study revealed that S-metolachlor in combination with oil palm frond residue (OFP) provided great suppression of herbicide-resistant biotype of goosegrass. However, study on interaction between OPF and S-metolachlor is still limited. Hence, combined phytotoxic effects of OFP and S-metolachlor were evaluated at the ratios of 50:50, 40:60, 30:70, 20:80 10:90 based on Additive Dose Model under glasshouse conditions. Pre-emergence application of S-metolachlor and OPF were able to reduce goosegrass emergence by 90% ($ED_{90}$) at 3.5 t ha$^{-1}$ and 148 g a.i. ha$^{-1}$, respectively. The $ED_{90}$ values of S-metolachlor were greatly reduced by approximately 72-92% when being incorporated with 1.0-1.4 t ha$^{-1}$ of OPF at 50:50 and 40:60 ratios. However, the interaction turned antagonism as the rate of OPF was increased from 3.0 to 4.0 t ha$^{-1}$ at the ratios of 30:70, 20:80 and 10:90. These results suggest that S-metolachlor in combination with OPF and applied as mulch could provide synergistic or antagonistic activity for goosegrass management depending on the ratio of oil palm frond residue combined with S-metolachlor.

**Key words:** Biostimulation, Growth, Milkweed, Sargassum, Spirulina and Seed vigor.

## Introduction

Goosegrass is a problematic annual weed, commonly found in vegetable farms, orchards, and immature oil palm plantations (Chuah & Ismail, 2010) and aerobic rice fields (Chauhan, 2012, Selvarajh *et al.*, 2018) and turfgrass (Wiecko, 2000). It is considered as one of five most troublesome weeds in the world which greatly affects 46 different crop species production in over 60 countries (Stecker, 2010) because its seedheads are present throughout the year (Wiecko, 2000). Herbicide application is a common practice utilized in farms and plantations to control the goosegrass infestation. Unfortunately, the intensive used of herbicide alone has resulted in the evolution of goosegrass resistance to various groups of herbicides such as paraquat, imidazolinones, glyphosate, metribuzin, glufosinate, aryloxyphenoxyproprionates, and cyclohexane (Heap, 2020). This incident had minimized the herbicide option for farmer to use for goosegrass control in farm.

S-metolachlor, an enantiomerically- enriched form of metolachlor (> 80% S-isomers) is one of the pre-emergence herbicides used to control grassy weeds and broadleaved weeds in orchard and vegetable farms (Peter *et al.*, 1998). To date, however, S-metalachlor-resistant weed biotype has not been documented yet (Heap, 2020). In order to slow down or reduce the evolution of herbicide resistance cases, multiple tactics should be employed to reduce selection pressure of the herbicide. For over a decade, cover crop and natural crop residue are used to control weed in early season, however it has been proven that the residue alone is not sufficient to provide full-season weed control (Teasdale *et al.*, 2003) and commercially acceptable level of weed control (Skroch *et al.*, 1992). Hence, a combination of more than one method should be practiced to provide an effective weed control, which had been demonstrated by Case & Mathers (2003) where the application of pre-emergence herbicide-treated mulches could extend the weed control over 300 days as compared to 45 days when ground herbicide was applied. Teasdale *et al.*, (2005) documented that, hairy vetch residues combined with 10-fold lower metolachlor rate provided a better suppression on weed emergence than single metolachlor application. Nevertheless, previous field research on the interaction between the herbicide and crop residue showed mixed results, from no interaction (Gallagher *et al.*, 2003) to antagonism (Chauhan & Abugho, 2012) or potential synergism (Teasdale *et al.*, 2005), depending on type and rate of herbicides and crop residues used.

Oil palm frond residues in combination with imazethapyr has been demonstrated to inhibit several weed species effectively in nurseries (Dilipkumar *et al.*, 2019, Nordin *et al.*, 2019) and coconut plantation (Dilipkumar *et al.*, 2017). Chuah *et al.*, (2018) revealed that 4 t ha$^{-1}$ oil palm frond residue powders in combination with reduced rate of S-metolachlor at 32 g ha$^{-1}$ provided suppression of glyphosate-resistant biotypes of goosegrass on an ambarella farm with more than 85% reduction of weed density and biomass, respectively, under field conditions. This result suggests that there could be a potential synergism between S-metolachlor and the oil palm frond residue. However, the exact joint action between S-metolachlor and oil palm frond residue has not been determined precisely. A model is required to predict dose responses to combinations of S-metolachlor and oil palm residue rate accurately and enable a clear identification of synergism. Therefore, this research aimed to determine whether there was a synergistic interaction between S-metolachlor and oil palm frond residues on emergence and early growth of goosegrass.

## Materials and Methods

**Herbicide:** S-metolachlor (Dual Gold 960 EC, 87.3% w/w, Sygenta Crop Protection, Selangor, Malaysia) was used in the experiments.

**Plant materials:** Fronds of oil palm (*Elaeis guineensis* var. Tanera) were sampled from an oil palm plantation in Setiu, Terengganu, Malaysia. The fronds were cleaned and sun-dried under full sunlight for a week. Then, the fronds were ground into residue form (< 2 mm) and stored in a chiller at 5°C before use. The goosegrass seeds were collected from roadsides of Gong Badak, Kuala Terengganu. Seed viability of goosegrass was examined to ensure the seeds had germination percentage higher than 90%. Seed coats of the goosegrass seeds were removed by using sand papers.

**Growth medium:** Kangkung series soil was collected from a coconut plantation at Agricultural Research and Development Institute of Malaysia (MARDI), Hilir Perak Station, Teluk Intan, Perak (3°53' N, 100°51' E). Soil samples were collected at 20 cm deep and transferred to a glasshouse. The soil was dried under sunlight, ground and sieved to pass through a 2-mm screen.

**Experiment 1: Single application of s-metolachlor or oil palm frond residue:** A total of 75 g Kangkong soil series was filled in each cup (5 cm diameter, 5 cm height) with four holes at the bottom. The cups were placed in a 15- by 10- by 8-cm tray and 100 mL water was applied daily to moist the soil surface. A total of 20 goosegrass seeds were sown evenly on the surface of soil in each cup. S-metolachlor was applied using micropipette (Eppendorf 10-1000 µl) at a series of equivalent rates viz., 0, 12.5, 25.0, 50.0, 100.0, or 200.0 g ai $ha^{-1}$ at a volume of 450 L $ha^{-1}$. OPF residue was applied evenly as mulches at different equivalent rates viz., 0, 0.75, 1.50, 3.00 or 6.00 t $ha^{-1}$, respectively. All treatments were applied on the soil surface one day after the seeds were sown under glasshouse conditions. Glasshouse conditions were maintained with relative humidity of 80%, a temperature of 35-38°C, and 12 h photoperiod at a light intensity of 750-1000 µ mol $m^{-2}$ $s^{-1}$. Number of seedling emergence was counted while shoot fresh weight of goosegrass seedling was measured 30 days after treatment (DAT). Each treatment was arranged in a completely randomized design with five replicates. Seedlings were considered emerged when the shoot lengths were >2 mm. The above ground living tissues remaining of each seedling were cut and weighted by using an electronic balance. The data were expressed as percentages of their respective controls as follows:

$$y = (xT/ xC) \times 100$$

where, y is shoot emergence rate or shoots fresh weight, xT is number of seeds with emerged shoots or shoot fresh weight in treatment, and xC is number of seeds with emerged shoots or shoots fresh weight in control.

**Experiment 2: Joint action between S-metolachlor and oil palm frond residue:** Oil palm frond residue in combination with S-metolachlor at the ratios of 50:50, 40:60, 30:70, 20:80, 10:90 and control (zero treatment) were prepared based on the single S-metolachlor/oil palm frond residue $ED_{50}$ value (rate that gives 50% inhibition) for goosegrass emergence in experiment 1. The OPF residues were treated with S-metolachlor using a compression sprayer (Matabi Style 7; Goizper, Bergara, Spain) equipped with flat-fan nozzles, calibrated to deliver a volume of 450 L $ha^{-1}$ at 200 kPa and dried for 24 hours under glasshouse conditions. Seventy-five gram of Kangkong series soil was filled in each cup with holes at the bottom for irrigation as described previously. Each ratio of S-metolachlor treated-oil palm frond mulch was applied evenly on soil surface one day after 20 goosegrass seeds were sown in the cup. Number of seedling emergence was counted while shoot fresh weight was determined at 30 DAT. The treatments were arranged in a completely randomized design with five replicates. The data were expressed as percentages of their respective controls as described above.

### Statistical analysis

To obtain application rate that gave 50% inhibition, the data from Experiment 1 were fitted to a logistic regression model (SigmaPlot 2006 version 10.0, Systat Software, Inc., 225 Washington St., Suite 425, Chicago, IL 60606), as follows (Kuk *et al*., 2002):

$$Y = d/ (1 + [x/ x^0]^{b})$$

where, Y is percentage of seedling emergence or shoot fresh weight of goosegrass, d is the coefficients corresponding to the upper asymptotes, x is rate of S-metolachlor / oil palm frond residue, $x^0$ is the rate of S-metolachlor /oil palm frond residue required to inhibit the seedling emergence/shoot fresh weight by 50% relative to untreated seedling, and b is the slope of the line. Regression analyses were conducted to calculate the S-metolachlor or oil palm frond residue rates required to reduce the shoot fresh weight or shoot emergence by 50%. T-test was conducted to compare the difference between two treatments in b values at 5% of significant level.

In the Experiment 2, combined phytotoxic effects of oil palm frond residue rate and S-metolachlor were evaluated based on the ADM model using seedling emergence and shoot fresh weight data. Isobolograms are used to show the S-metolachlor-treated oil palm frond mulch needed to produce a 90% effect level in a test system. Data were analyzed based on the method described by Sorensen *et al*., (2007) using sigmoid log-logistic dose-response model as follows:

$$y_i = d/1 + \exp \{2b_i [\log (e_i) + 1.099/b_i - \log (x_i)]\}$$

where, $y_i$ is the response for the $_i$ th S-metolachlor or oil palm frond residue rate $x_i$ (i = 1…., 5), and d is the common upper limit of the response of all mixture ratios when the oil palm frond residue or S-metolachlor rate $x_i$ is zero. The parameter $e_i$ is the rate of oil palm frond mulch and S-metolachlor $i$ giving a response of 90% of d, and $b_i$ is proportional to the slope around $e_i$.

In the second model, the $e_i$ values of the first model were constrained to follow an isobole model proposed by Volund (1992), which includes two parameters (η1 and η2) to describe the isobole curvature as follows:

$$\left(\frac{x_1}{ED_1}\right)^{\eta_1}\left(\frac{x_1}{ED_1}+\frac{x_2}{ED_2}\right)^{1-\eta_1}+\left(\frac{x_2}{ED_2}\right)^{\eta_2}\left(\frac{x_1}{ED_1}+\frac{x_2}{ED_2}\right)^{1-\eta_2}=1$$

where x is the rate of oil palm frond mulch and S-metolachlor at a predefined effect level, and *ED* is the rate of the same oil palm frond mulch or S-metolachlor giving that effect, when tested alone. The subscription 1 and 2 denote the oil palm frond mulch and S-metolachlor in the mixture.

The third model, which replaces ei with an isobole model, was equal to the second model with η1 and η2 fixed at the value 1. One-sample T test was used to determine deviation from ADM based on third model values at 5% of significant level. The sum of toxic units (ΣTU) signifies the relative amount of chemical in a mixture that will give a certain effect. This study works with a 90% effect level. The ΣTU50:50 is calculated as $1/(2^{-\eta 1}+2^{-\eta 2})$ (Sorensen *et al*., 2007).

## Results

**Single application of S-metolachlor or oil palm frond residue:** The rates required for 50% reduction ($ED_{50}$) of the seedling emergence and growth ranged from 2.5 to 2.6 t $ha^{-1}$ for OPF mulches while the S-metolachlor rate needed for $ED_{50}$ of the seedling emergence and growth were 30.4 and 9.4 g a.i. $ha^{-1}$, respectively (Table 1). The slopes of regression lines of S-metolachlor and oil palm residues are represented by *b* values. Overall, there was a significant difference between S-metolachlor and OFF in the respective *b* values of goosegrass seedling emergence and growth ($p \leq 0.05$).

**Table 1. The $ED_{50}$ and *b* values of *Eleusine indica* in relation to S-metolachlor and oil palm frond mulch.**

| Parameter | S-metolachlor | Oil palm frond |
|---|---|---|
| | $^{a}ED_{50}$ | |
| Seedling emergence | 30.42 (1.51) | 2.51 (0.01) |
| Shoot fresh weight | 9.41(2.29) | 2.60 (0.25) |
| | #*b* value | |
| Seedling emergence | 1.39 (0.08) | 6.38 (0.13) * |
| Shoot fresh weight | 1.30 (0.36) | 4.40 (1.80) * |

Note:$^{a}ED_{50}$ is the rate of S-metolachlor (g ai $ha^{-1}$) or rate of oil palm frond mulch (t $ha^{-1}$) required to reduce seedling emergence and shoot fresh weight of goosegrass by 50%

#*b* is slope of regression line.

**b* value oil palm frond mulch differs significantly from that of S-metolachlor at $p \leq 0.05$. The values in parentheses are the standard errors of means

**Table 2. $^{a}\eta_1$, $^{b}\eta_2$ and $^{c}\Sigma TU_{50:50}$ values of goosegrass in relation to mixture of S-metolachlor plus oil palm frond residue.**

| Combination | S-metolachlor plus oil palm frond residue | |
|---|---|---|
| | Emergence | Shoot fresh weight |
| $\eta_1$ | 0.495 (0.062) | 1.450 (0.100) |
| $\eta_2$ | 0.065 (0.002) | 0.089 (0.010) |
| $\Sigma TU_{50:50}$ | 0.600 (0.014) | 0.765 (0.022) |

$^{a}\eta_1$ and $^{b}\eta_2$ describe the isobole curvature. $^{c}\Sigma TU_{50:50}$ is the sum of toxic unit that signifies the relative amount of chemical in a mixture that gives 50% effect. The values in parentheses are the standard deviations of the means (n = 3)

**Joint action between S-metolachlor and oil palm frond residue:** It is noted that synergism was observed when oil palm frond residue was combined with S-metolachlor as pre-emergence application. The synergistic effect was found at the ratio of 50% S-metolachlor + 50% oil palm frond residue on goosegrass seedling emergence while the synergism on goosegrass shoot fresh weight was observed at the ratio of 40% S-metolachlor + 60% oil palm frond residue. According to the previous experiment, single application of S-metolachlor at the rate of 148 and 51 g ai $ha^{-1}$ were required to inhibit goosegrass emergence and growth by 90% ($ED_{90}$), respectively (Figs. 1 & 2). However, when combined with oil palm frond residue, the combination at the rate of 12.3 g ai $ha^{-1}$ S-metolachlor plus 1.0 t $ha^{-1}$ oil palm frond mulches and 10.8 g ai $ha^{-1}$ S-metolachlor plus 1.4 t $ha^{-1}$ oil palm frond mulches at the ratio of 50:50 and 40:60 respectively were found to achieve the same inhibitory effect on goosegrass seedling emergence and shoot fresh weight, indicating that, S-metolachlor can be reduced by approximately 79-92 % (Fig. 3). However, the synergistic effect was reduced and turned antagonism as the rate of oil palm frond residue was increased from 3.0 to 4.0 t $ha^{-1}$ as shown at the ratios of 20% S-metolachlor + 80% oil palm frond residue and 10% S-metolachlor + 90% oil palm frond residue (Fig. 3).

According to one sample T-test, the combination of S-metolachlor and oil palm frond residue deviated from ADM except the ratios of 40% S-metolachlor + 60% oil palm frond residue and 30% S-metolachlor + 70% oil palm frond residue for goosegrass seedling emergence and at a ratio of 50% S-metolachlor + 50% oil palm frond residue for shoot fresh weight. $\Sigma TU_{50:50}$ for S-metolachlor plus oil palm frond residue in goosegrass emergence are 0.60, implying that about 60 % of S-metolachlor-treated oil palm frond is needed, to provide 90% inhibition as compared with that expected from ADM.

Likewise, $\Sigma TU_{50:50}$ for S-metolachlor in combination with oil palm frond residue in goosegrass shoot fresh weights is 0.77, indicating that about 77% of S-metolachlor-treated oil palm frond is required to reduce the shoot fresh weight by 90% as compared to the expected from ADM (Table 2). These results suggest that synergistic effect of S-metolachlor and oil palm frond residue combination is approximately 15% more apparent in seedling emergence than shoot fresh weight.

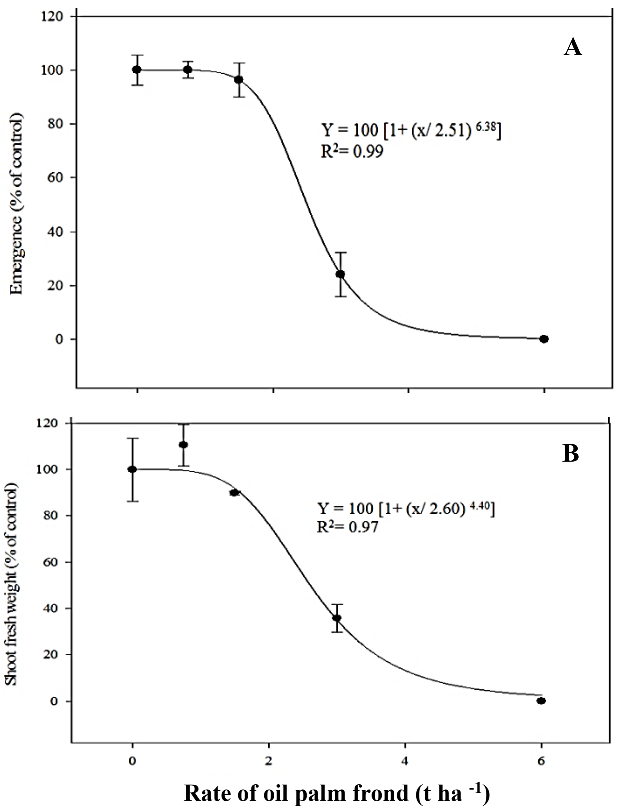


Fig. 1. Pre-emergence applications of oil palm frond mulch on the seedling emergence (A) and shoot fresh weight (B) of goosegrass one month after treatment. Vertical bars represent standard deviation (SD) of the mean.

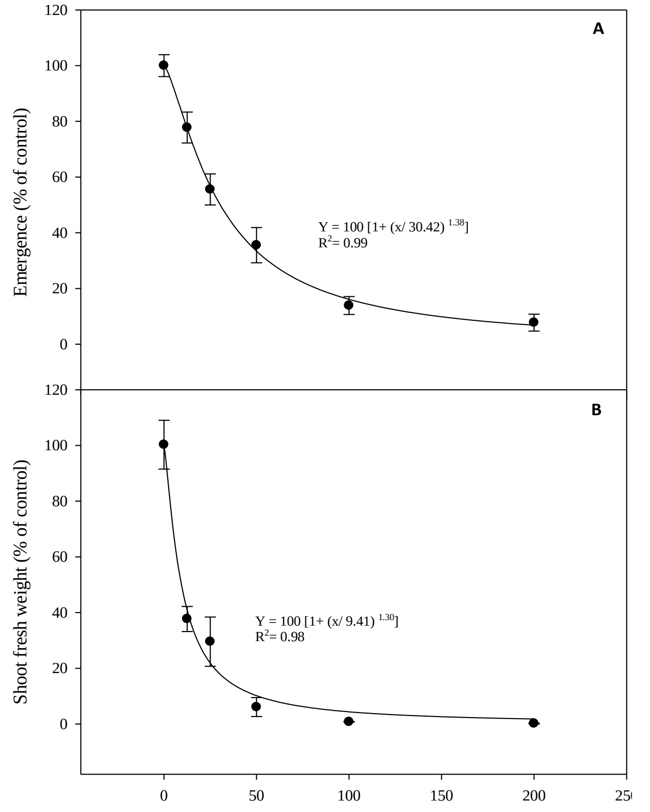


Fig. 2. Pre-emergence applications of S-metolachlor on the seedling emergence (A) and shoot fresh weight (B) of goosegrass one month after treatment. Vertical bars represent standard deviation (SD) of the mean.

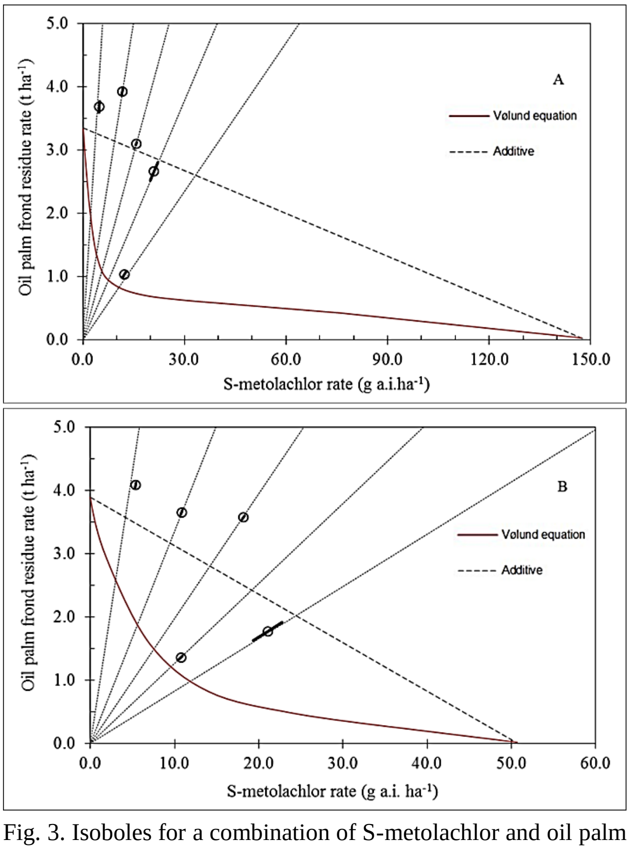


Fig. 3. Isoboles for a combination of S-metolachlor and oil palm frond residue on goosegrass seedling emergence (A) and shoot fresh weight (B), at the $ED_{90}$ level, with data given as $ED_{90}$ ± standard error.

## Discussion

Strong synergism was observed when S-metolachlor and oil palm frond residue were combined and applied as mulch at the ratios of 50:50 and 40:60. The result of current study is in agreement with the finding of Teasdale *et al.*, (2005), in which 5 t ha$^{-1}$ of hairy vetch residue (*Vicia villosa* Roth) and 10 g ha$^{-1}$ of S-metolachlor were found to reduce 13 and 16% of smooth pigweed emergence, respectively. Interestingly, the combination of both treatments devoted a synergistic interaction by reducing 86% of smooth pigweed emergence compared to single application of S-metolachlor which was needed approximately 1000 g ai ha$^{-1}$ to achieve the same inhibitory effect. On the other hand, the synergism between hairy vetch residue and S-metolachlor occurred at the highest rate, 6 t ha$^{-1}$ and 1000 g ai ha$^{-1}$, respectively, where velvetleaf emergence was reduced by 70% compared with 33 and 8% reduction of hairy vetch residue and S-metolachlor alone, respectively.

There are several possible mechanisms which explain the synergistic action between oil palm residue of frond and S-metolachlor when combined together and applied as mulch. Firstly, a significant difference was observed between the b values of S-metolachlor and oil palm residues of fronds when being fitted into log logistic regression (Table 1), implying S-metolachlor and the oil palm fronds have different mode of actions. This finding suggests that S-metolachlor and oil palm residues may exhibit different mode of actions in suppressing goosegrass emergence and growth, thus providing synergistic action when S-metolachlor was combined with the oil palm residues of frond and applied as mulch.

Secondly, it has been documented that plant lignin can act as a matrix to control the release of herbicides such as diuron and 2, 4 D (Oliveira *et al*., 2000). The presence of lignin, an essential constituent of woody plant cell walls, was found to be the best herbicide carrier for controlled release of herbicide (Oliveira *et al*., 2000). Derr (1994) stated that the combination of herbicide with different carriers can help reduce the amount of herbicide needed, enhance and/or extend efficacy on weed control. Organic mulches have been proven to be the effective carriers for herbicide (Case & Mathers, 2006; Mathers, 2003; Mathers & Case, 2010). Oil palm residue of frond with 20.6% lignin content (Lai & Idris, 2013) might make it a good candidate as slow release carrier for S-metolachlor. Hence, the leaching potential of S-metolachlor with water solubility of 530 mg $L^{-1}$ and organic carbon absorption coefficient ($K_{oc}$) of 200 mL $g^{-1}$ can be reduced when applied onto soil (Rivard, 2003). Furthermore, Knight *et al*., (2001) have reported that application of pre-emergence herbicide-treated mulches reduced the herbicide leaching potential by 35-75% compared with the bare soil herbicide application method. On the other hand, Tharayil *et al*., (2006) claimed that competition for sorption sites occurs if more than one non-identical molecule can occupy the same sites. Thus, allelochemicals released by the oil palm residues and S-metolachlor may be competing for the same sorption sites in the soil. As a result, free S-metolachlor molecules are more available for uptake of goosegrass seedling.

During 1970 and 1980s, some innovative techniques were created to improve the performance of metolachlor for weed control. Tablet formulation by combining 4% metolachlor in calcium phosphate controlled Italian ryegrass for up to 14 months in 1-gal containers (Verma & Smith, 1981). The tablet with 40 kg $ha^{-1}$ metolachlor was found to provide 80% suppression of annual ryegrass growth and caused less injury to 'Cranberry' cotoneaster while the granular form of metolachlor only reduced shoot fresh weight of ryegrass by 10% at 150 days after treatment (Koncal *et al*., 1981). Meanwhile, metolachlor with granular formulation applied at the rate of 40 kg $ha^{-1}$ showed a greater leaching potential as compared to metolachlor appeared in tablet formulation. A bioassay study further revealed that granular metolachlor at 40 kg $ha^{-1}$ leached to a depth of 10-12.5 cm, while metolachlor at the same rate in slow release tablet form was present primarily in 0-7.5 cm (Verma & Smith, 1981), indicating the important role of herbicide carrier in reducing the leaching potential in soil.

Alternatively, the enhancement of S-metolachlor activity by the oil palm residue may be due to the etiolating effect of the plant residue on emerging seedlings. An emerging seedling must expand widely or through mulching materials to gain more resources such as radiation before seed reserves are exhausted. Teasdale & Mohler (2000) stated that light prevention by mulching is the major contribution on suppressing weed emergence. A study has shown that hypocotyl elongation of canola seedlings in reduced light led to reallocation of carbon and nutrient resources away from leaves and roots, thereby reducing the growth development of plants (Bruce, 2003).

## Conclusion

Oil palm frond residue has potential to be combined with S-metolachlor to produce synergistic activity at the ratios of 50:50 and 40:60. However, the interaction could turn antagonism with an increase of oil palm frond ratio from 70% to 90% when mixed with S-metolachlor. This finding implies that the ratio of oil palm frond residue in combination with S-metolachlor is an important role in affecting the degree of phytotoxicity for weed management. Further study is being carried out to elucidate mechanism of actions which lead to synergistic and antagonistic activity.

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