https://doi.org/10.26651/allelo.j/2020-51-2-1300 0971-4693/94 Euro 20.00
Allelopathy Journal 51 (1): 195-208 (September, 2020) *International Allelopathy Foundation* 2020
Table: 2, Figs : 5

Herbicidal activity of allelochemical 2,4-di-tert-butylphenol on weeds (*Asystasia gangetica, Eleusine indica, Leptochloa chinensis* and *Oldenlandia verticillata*)

M.Z. Norhafizah[1], W.S. Wan Nur Suzani Sazleen[2] and T.S. Chuah*
Faculty of Plantation and Agro-technology,
University of Technology Mara, 02600 Arau Perlis, Malaysia
E. Mail: chuahts@uitm.edu.my

(Received in Revised Form: July 13, 2020)

ABSTRACT

This glasshouse study aimed to determine the herbicidal activity of allelochemicals 2,4-di-tert-butylphenol (2,4-DTBP) on test weeds (*Asystasia gangetica, Eleusine indica, Leptochloa chinensis* and *Oldenlandia verticillata*) and to find the tolerance of test crops (*Brassica rapa, Oryza sativa, Zea mays*) to 2,4-DTBP. The 2,4-DTBP applied at 7 kg ai/ha inhibited the weeds seedling growth by 15-40 % only, showing its poor post emergence herbicidal activity. In contrast, soil bioassay confirmed pre-emergence herbicidal activity of 2,4-DTBP that reduced the seedling growth of weeds (*L. chinensis*, *E. indica* and *O. verticillata*) by 50- 80 % at 2.5 kg ai/ha but *A. gangetica* was tolerant to 2,4-DTBP even at 5 kg ai/ha. Crop tolerance test revealed that *Zea mays* was tolerant to 2,4-DTBP. In contrast, root lengths of transplanted *Brassica rapa* seedlings and *Oryza sativa* seedlings were reduced by 10- 15 % at 2,4-DTBP doses of 1.25 to 5 kg ai/ha, 14 days after treatment. However, these two crops seedlings became tolerant to 2,4-DTBP at 5 kg ai/ha, when applied 4 days after transplanting. Thus 2,4-DTBP may be developed as novel pre-emergence natural herbicide, without injuring the crops depending on their growth stages and crop species.

Key words: Allelochemical, 2,4-di-tert-butylphenol, 2,4-DTBP, *Asystasia gangetica, Brassica rapa,* crops, *Eleusine indica,* Herbicidal activity, *Leptochloa chinensis,* maize, *Oldenlandia verticillata, Oryza sativa,* weeds, *Zea mays*

INTRODUCTION

The plant allelochemicals play role in weed invasion (23). In plants, the allelochemicals are present in the leaves, bark, roots, root exudates, flowers and fruits (38). The allelochemicals after their release in environment suppresses or stimulates the growth of plants is called allelopathy (18). These allelochemicals improves the crop productivity and protects the environmental by eco-friendly control of pests (weeds, pests, crop diseases) (4,19). The allelochemicals 2,4-di-tert-butylphenol (2,4-DTBP) is insoluble in water (12) and is synthesized through alkylation of phenols. The phenols are found in dicot (107 species, 58 families) and monocot (22 species, 8 families) plants. 2,4-DTBP was detected in methanolic extracts of cones and bark of *Pinus yunnanensis* Franch. (20) and in n-hexane extracts of the cones of *Pinus kesiya* var. *langbianensis* (A. Chev.) Gaussen ex Bui (26). The GC-MS analysis showed that 2,4-DTBP is major component in water extracts of fresh needles of *Pinus tabulaeformis* Carr. (36). It is also present in medicinal plants such as *Gynura cusimbua* D. Don S. Moore (32) and *Plumbago zeylanica* L. (1).

*Correspondence author, [1]Faculty of Agro-based Industry, University of Malaysia Kelantan, Jeli Campus, 17600, Jeli, Kelantan, Malaysia; [2]School of Food Science and Technology, University of Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia.

The allelochemicals 2,4-DTBP is allelopathic also in soilless conditions (5). The allelochemical 2,4-DTBP extracted from the *Chrysanthemum indicum* inhibited the seed germination and seedling growth of lettuce (*Lactuca sativa* var. ramosa Hort.) and rapeseed (*Brassica napus* L.) (16). The invasiveness of *Imperata cylindrical* (L.) P. Beauv. (39) and *Pennisetum purpureum* Schum. (5) was due to the presence of 2,4-DTBP. The 2,4-DTBP at 0.1 mg/mL, extracted from the rhizome of *I. cylindrical* inhibited the root and shoot growth of weeds [beggar ticks (*Bidens pilosa* L.), leucaena (*Leucaena leucocaphala* L. de Wit) and barnyardgrass (*Echinochloa crus-galli* (L.) Beauv) (39)]. It reduced the shoot growth of several weeds by 50 %. The 2,4-DTBP concentration was 50 and 200 µg/mL in the culm and leaf of napier grass (*P. purpureum*) (7,10). It caused the wilting of lamina, necrosis, and shortened the root hairs than non-treated plants. Besides, it also induces oxidative stress by generation of reactive oxygen species, causing lipid peroxidation, membrane damage, reducing the chlorophyll content, chlorophyll fluorescence, transpiration, and net photosynthetic rate in the leaves of weeds (7,10,13,14).

However, there is limited information about the use of allelochemical 2,4-DTBP as herbicide. Hence, this study aimed to evaluate the pre- and post- herbicidal activity of 2,4-DTBP and its effects on test crops. We studied the phytotoxic effects of allelochemicals 2,4-DTBP on test grasses (*Leptochloa chinensis* and *Eleusine indica*) and broadleaves weeds (*Oldenlandia verticillata* and *Asystasia gangetica*) due to their invasiveness and high abundance in paddy fields, oil palm plantations, corn and vegetables farms.

MATERIALS AND METHODS

The post- and pre- emergence herbicidal studies were done during May-July 2018, at University of Malaysia Terengganu [Latitude 5.41° N, Longitude 103.09° E], 15 m above sea level, and annual rainfall: 2988 mm. The experimental treatments consisted of 4-factors; (i). Test weeds 4 (*Asystasia gangetica, Eleusine indica, Leptochloa chinensis* and *Oldenlandia verticillata)*, (ii). Test crops 3 *(Brassica rapa, Oryza sativa, Zea mays*), (iii). Stages of 2,4-DTBP application (Pre-, Post-emergence) and (iv). 2,4-DTBP doses. In this study, 3- separate Experiments were done, (i) Post-emergence Application [2 Factors: 2,4-DTBP doses 4 (7, 14, 28, 56 kg ai/ha) and 4- test weed spp], (ii) Pre-emegence Application [2 Factors: 2,4-DTBP doses 4 (0.625, 1.25, 2.5, 5.0 kg ai/ha) and 4-test weed spp] and (iii). Crops tolerance test [3 Factors: 2,4-DTBP doses 3 (1.25, 2.5, 5.0 kg ai/ha) growth stages 4 (0,2,4,6 days after crop sowing/ transplanting) and 3-test crops]. The treatments were replicated 5-Times in Complete Randomised Design.

I. Chemicals: 2,4-di-tert-butylphenol (99 % purity) and dimethylsulfoxide (DMSO) were purchased from Sigma Chem. Co., Kuala Lumpur, whereas, saponin (69 % purity), a natural byproduct of tea oil (*Camellia oleifera* Abel.) was purchased from Hangzhou Choisun Tea Sci-Tech Co. Ltd.

II. Seeds: The test weeds and crops seeds were obtained as per Table 1.

Seeds treatment: The seeds of *E. indica* and *O. verticillata* were scarified to remove the seed coats using sand paper. The naked seeds were soaked in 0.2 % K_2No_3 solution for 24 h to break seed dormancy. The viability of all weed seeds was > 90 %.

Table 1. Seed collection sites of bioassay species

No.	Name	Collection site
	Weed species	
1	*Leptochloa chinensis*	Rice fields of Pengkalan Maras, Kuala Nerus, Terengganu
2	*Eleusine indica*	Wasteland of Gong Badak, Kuala Nerus, Terengganu
3	*Oldenlandia verticillata*	Oil palm plantation Felda Gerdong, Kuala Berang, Terengganu
4	*Asystasia gangetica*	
	Crops	
1	*Brassica rapa* L.	Chiap Hup Agriculture Development Sdn. Bhd, Tangkak, Johor
2	*Oryza sativa* L. (MR219)	Pejabat Peladang Bukit Bayas, Kuala Terengganu, Terengganu
3	*Zea mays* L.	Soon Huat Seeds Co. Sdn Bhd., George Town, Penang

III. Cup Culture Glasshouse assays

(i). Soil: Renggam soil series, characterized as silt loam soil (18 % clay, 59 % silt, and 25 % sand), was collected from Kampung Maras, Kuala Nerus, Terengganu (5º4’ N, 103º5’ E). The physico-chemicals and microbial analyses of Renggam soil series is shown in Table 2. Soil samples were collected up to 20 cm depth and transferred to glasshouse. The soil was sun-dried, ground and sieved to pass through a 2-mm screen. The bioassay was done in paper cups (4.5 cm dia, 7 cm depth with 5 holes at the bottom) and each cup was filled with 50 g Renggam soil. These cups were kept in glasshouse (Relative humidity: 78-80 %, temperature of 28-30 ˚C and 12 h photoperiod [photosynthetic photon flux density (PPFI) of 800]).

Table 2. Soil physico-chemicals properties and bacterial count of soil used in studies

Parameter	Physico-chemicals properties
Soil texture	Silt loam
Clay (%)	18
Silt (%)	59
Sand (%)	25
Organic carbon (%)	1.7
Cation exchange capacity (meq/100)	0.7
pH	4.58
Nitrogen (N) (%)	0.2
Phosphorus (P) (mg/kg)	53.78
Potassium (K) (mg/kg)	836.42
Total bacterial count, (CFU g^{-1})	1.5 x 10^3

Meq: milliequivalents, CFU: colony forming units.

(ii). Post-emergence herbicidal test: One seed of each bioassay species was sown in each paper cup. The cups were irrigated daily with 25 mL water per cup to keep the soil moist. The 2,4-DTBP doses were 0, 7, 14, 28 and 56 kg ai/ha; these were prepared by dissolving the compound in 0.3 % dimethylsulfoxide (DMSO) and 0.03 % saponin. The mixture was stirred until the 2,4-DTBP powder was dissolved completely. Non-treated cups (Control) were applied only with 0.3 % DMSO and 0.03 % saponin. The seedlings of each bioassay species at 2-leaf stage were sprayed at 800 µl of liquid per cup (450 L ha^{-1}) with hand sprayer. After 21 days of treatments, the aboveground parts of plants were harvested. Shoot fresh weight and root length of seedlings were determined and expressed as % of control.

(iii). Pre-emergence herbicidal test: Twenty-five seeds of test weeds (*L. chinensis*, *E. indica*, *O. verticillata*) and 5- seeds of *A. gangetica* were sown separately in each paper cup with 50 g soil. The 2,4-DTBP herbicide was dissolved in 0.24 % DMSO and 0.024 % saponin to prepare application rates of 0.625, 1.25, 2.5 and 5 kg ai/ha because these rates were much lower than post-emergence herbicidal test (6-56 kg ai/ha). Non-treated cups (Control) were applied only with 0.24 % DMSO and 0.024 % saponin. One to 3- days after seeds sowing (depending on test bioassay species), the herbicide doses were applied on the soil surface with micropipette (Eppendorf 10-1000 µl) to provide 800 µl liquid per cup. The seedlings were considered emerged, after attaining plumule length of 2 mm above ground. After 21 days of treatments, the emerged seedlings were counted, the seedlings root length and shoot fresh weight were recorded and expressed as % of control.

(iv). Crop tolerance test: The herbicide 2,4-DTBP was dissolved in 0.24 % DMSO and 0.024 % saponin to prepare 3- doses: 1.25, 2.5 and 5.0 kg ai/ha. These were applied to *Z. mays*, *B. rapa* and *O. sativ*a seedlings at 0, 2, 4 and 6 days after transplanting (*B. rapa* and *O. sativ*a) or after sowing (*Z. mays*). One *Z. mays* seed was sown per paper cup filled with 75 g soil. The seeds of *B. rapa* were sown in potting mix medium in seedling tray. One seedling (2 to 3-leaf stage) was transplanted into each cup containing 75 g soil. For *O. sativa*, the seeds were soaked for 6 h and then kept in wet paper towel for 42 h to induce seeds germination. One pre-germinated rice seed was transplanted into each cup with 100 g soil. Five days after application of 2,4-DTBP, water was filled in the cups (3 cm above the soil surface). Non-treated crop seedlings were used as control. The shoot fresh weight and root length of 3-test crops were recorded 14 days after the 2,4-DTBP treatment.

IV. Statistical analysis

The percentage data (shoot fresh weight and root length) of post emergence, pre-emergence herbicidal tests, and crop tolerance test were checked for homogeneity of variance and normality. Descriptive statistics were carried out to obtain mean and standard deviation of each treatment. For post-emergence and pre-emergence herbicidal tests, log10 transformation and square root (x + 0.5) transformation were performed, respectively, for the data of shoot fresh weight and root length before being subjected to two-way ANOVA. Meanwhile, the percentage data of crop tolerance test were subjected to three-way ANOVA without data transformation. The Tukey test was used to compare the mean among the treatments at 5 % of significant level. Differences were regarded as significant when the *P*-values were ≤ 0.05.

RESULTS AND DISCUSSION

POST EMERGENCE HERBICIDAL TEST

Shoot Growth: There was significant interaction between the fresh weight of weed species shoot and 2,4-DTBP dose. The effects of allelochemical 2,4-DTBP on shoot fresh weight of all test weed species varied with application rates (Figure 1), indicating that 2,4-DTBP significantly inhibited the shoot growth. At the lowest dose of 7 kg ai/ha, *E. indica* was more susceptible and its shoot growth was significantly suppressed (39 %) than *L. chinensis* and *O. verticillata* with 22 and 15 % inhibition, respectively. The highest dose of 56 kg ai/ha was most inhibitory (80 %) to shoot fresh weight of *L. chinensis* and *E. indica* as compared to only 60 and 45 % reduction in shoot fresh weight in *O. verticillata* and *A. gangetica*, respectively. However, allelochemical 2,4-DTBP application did not affect the weeds root length (Figure 2 Pooled data). The root growth of *L. chinensis* was drastically inhibited (62 %) by 2,4-DTBP application. In contrast, the root lengths of *E. indica*, *O. verticillata* and *A. gangetica* were reduced only by 25 % (Figure 2A), suggesting that root lengths of these 3- test weed species seedlings were less affected by 2,4-DTBP. In addition, the allelochemical 2,4-DTBP moderately reduced the root length of tested weed species (37-43 %) at 14- 56 kg ai/ha doses (Figure 2B).

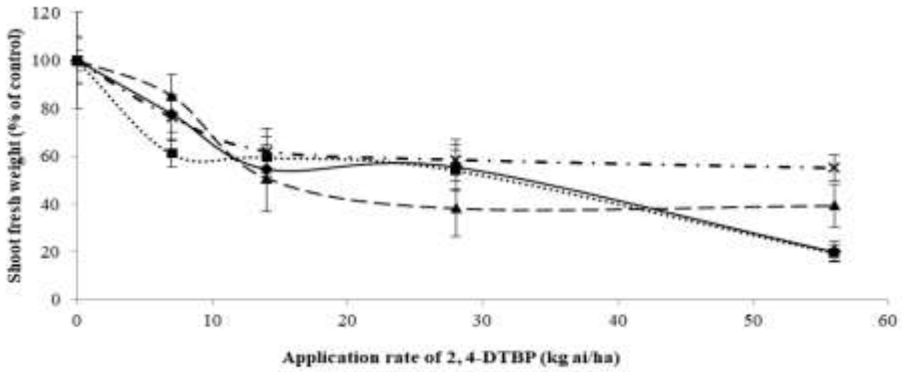

Figure 1. Post emergence application of 2,4-di-tert-butylphenol (2,4-DTBP) on shoot fresh weight of *Leptochloa chinensis* ( ), *Eleusine indica* ( ), *Oldenlandia verticillata* ( ) and *Asystasia gangetica* ( ) 21 days after treatment. Vertical bar represents standard deviation (SD) of the mean.

The 2,4-DTBP reduced the shoot fresh weight of test weeds seedlings at the lowest dose of 7 kg ai/ha (Figure 1). The Tricolorin A, (a natural compound derived from *Ipomoea tricolor* Cav) applied at 60 µM reduced the dry weight of *Trifolium alexandrinum* L. and *Physalis ixocarpa* Brot. by 37 % and 22 % respectively when treated (25). Interestingly, 2,4-DTBP at 7 kg ai/ha (equivalent to 70 µM) also reduced the shoot weight of *E. indica* and *L. chinensis* by 39 and 22 %, respectively. The seedlings of tested species showed retarded growth due to chlorosis, necrosis and desiccation of leaves. This finding is in accordance with Yang *et al*. (40), where the exogenous application of phenolic compounds (ferulic acid, p-coumaric acid and o-hydroxyphenyl acetic acid) degraded the chlorophyll in rice leaves. The 1,8-cineole (an essential oil), reduces the chlorophyll content and cellular respiration of weed, *Ageratum conyzoides* (35). Heisey (15) also stated that post application of ailanthone (a natural compound isolated from *Ailanthus altissima* Mill), at the lowest concentration (0.5 kg ai/ha), completely inhibited the growth of 4-to 6-day old seedlings of *Lepidium sativum* L. *Amaranthus retroflexus* L. and *E. crus-galli*, besides, caused moderate mortality of *Abutilon theophrasti* Medik. at the highest rate of 8 kg ai/ha, within 5 days after treatment.

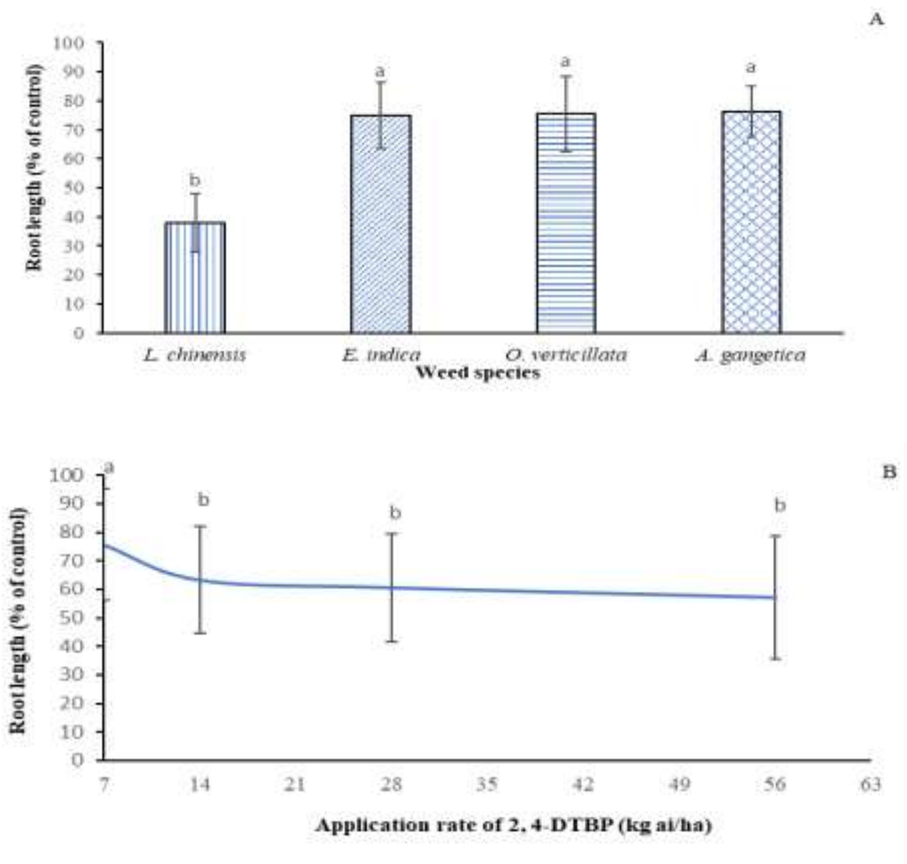

Figure 2. Main effects of weed species (A) and post emergence application rate (B) of 2,4-di-tert-butylphenol (2,4-DTBP) on root lengths of *Leptochloa chinensis*, *Eleusine indica*, *Oldenlandia verticillata* and *Asystasia gangetica* seedlings, 21 days after treatment. Vertical bar represents standard deviation (SD) of the mean. Main effect means followed by the similar letter has no significant difference at $p \leq 0.05$ after determined by Tukey test.

The weed species are susceptible to a single herbicide active ingredient and its dose of application. For example, cyhalofop-butyl herbicide at 0.2 kg ai/ha reduced the shoot fresh weight of *L. chinensis* by 80-90 %, while propanil at 2 kg ai/ha caused 85 % inhibition (31). On the other hand, glyphosate and paraquat herbicides completely controlled the weed *O. verticillata* seedlings at 0.80 kg ai/ha (6). These studies showed that the effective rate of synthetic herbicides to control the tested weed species, was < 2 kg ai/ha regardless of its active ingredients. However, the allelochemical 2,4-DTBP was required at high dose of 56 kg ai/ha to suppress 80 % weeds (*L. chinensis* and *E. indica*) seedlings growth, whereas, this dose reduced the seedling growths (40-60 %) of *O. verticillata* and *A. gangetica*. In general, the foliar application did not inhibit the root growth if the herbicide is not pre-emergence. Surprisingly, the allelochemical 2,4-DTBP treatment inhibited the root growths (25 %) of all tested weed species, while roots of *L. chinensis* were most sensitive to possessed the pre-emergence herbicidal activity.

PRE EMERGENCE HERBICIDAL TEST

(i). **Seedlings Emergence:** There were interactions between the allelochemical 2,4-DTBP dose and weed species emergence and seedling growth (shoot fresh weight and root length). The increased dose of 2,4 DTBP decreased the emergence of *L. chinensis*, *E. indica* and *O. verticillata* (Figure 3A). *L. chinensis* was the most sensitive (53 %) reduction at 2.5 kg ai/ha and 67 % reduction at 5 kg ai/ha. While, both *E. indica* and *O. verticillata* emergence were inhibited by 12 % at 2.5 kg ai/ha 2,4-DTBP. On the other hand, *A. gangetica* was most tolerant, because its emergence remained 100 % even at the highest dose of 2,4-DTBP.

(ii). **Shoot Growth:** The allelochemical 2,4-DTBP reduced the shoot fresh weight of tested weeds in dose dependent manner and the phytotoxicity was significant on *O. verticillata*, *L. chinensis*, *E. indica* than *A. gangetica* (Figure 3B). At the lowest dose of 0.625 kg ai/ha, the shoot growths of *O. verticillata*, *L. chinensis* and *E. indica* were inhibited only by 6-14 %. Interestingly at 2.5 and 5.0 kg ai/ha, the reduction in shoot growth was 40-58 % and 48-73 %, respectively. However, shoot fresh weight of *A. gangetica* seedling was reduced only by 10 % at 2,4-DTBP dose of 5 kg ai/ha.

Application of allelochemical 2,4-DTBP at 2.5 kg ai/ha reduced the shoot growth of grassy weeds (*L. chinensis* and *E. indica*) by 47 and 40 %, respectively (Figure 2B). Chuah *et al*. (9) also revealed that chilli leaf and stem extracts inhibited the seedling growth of *E. indica* by 62 and 45 %, respectively, at 40 g L^{-1}. Nazemi *et al*. (28) reported that coumarin at 3.8 kg ai/ha inhibited the shoot growth of grassy weed, *Sorghum halepense* L. Pers. by 50 %. On the other hand, the 2, 4-DTBP at 2.5 kg ai/ha reduced shoot growth (58 %) of broadleaf weed, *O. verticillata*. In comparison with 2,4-DTBP, sorgoleone exhibited greater herbicidal activity, it suppressed the shoot growth of *Rumex japonicas* Houtt., *Plantago asiatica, Portulaca oleracea* L. and *Eclipta alba* L. by 70-80 % at dose of 0.4 kg ai/ha (37). High efficacy of sorgoleone on the bioassay species may be due to its wettable powder formulation than soluble concentrate formulation of 2,4-DTBP. Uddin *et al*. (37) used kaolin and silicon dioxide (mineral clay and silica) as slow release carriers for sorgoleone. In addition, polyoxyethylene monooctadecyl ether [or its IUPAC name as 2-octadecoxyethanol] (2) was used as bio-surfactant (27) for sorgoleone to enhance its efficacy because it was exuded as oily droplet from the root hairs of sorghum (11).

(iii). **Root Growth:** The root lengths of *L. chinensis*, *E. indica* and *O. verticillata* were 83 – 88 % of non-treated control at dose of 0.625 kg ai/ha and decreased progressively with increased rate of application (Figure 3C). At 2.5 kg ai/ha of 2,4-DTBP the root lengths of *L. chinensis*, *O. verticillata* and *E. indica* seedlings reduced by 22, 44 and 53 %, respectively over non-treated control. These results imply that *L. chinensis* is the most susceptible to 2,4-DTBP treatment. On the other hand, root length of *A. gangetica* seedling was least affected by the application rates. Although the allelochemical 2,4-DTBP suppressed the emergence, shoot and root growth of *L. chinensis*, *E. indica* and *O. verticillata* effectively at 2.5 kg ai/ha, it failed to provide complete control even at the highest dose of 5 kg ai/ha.

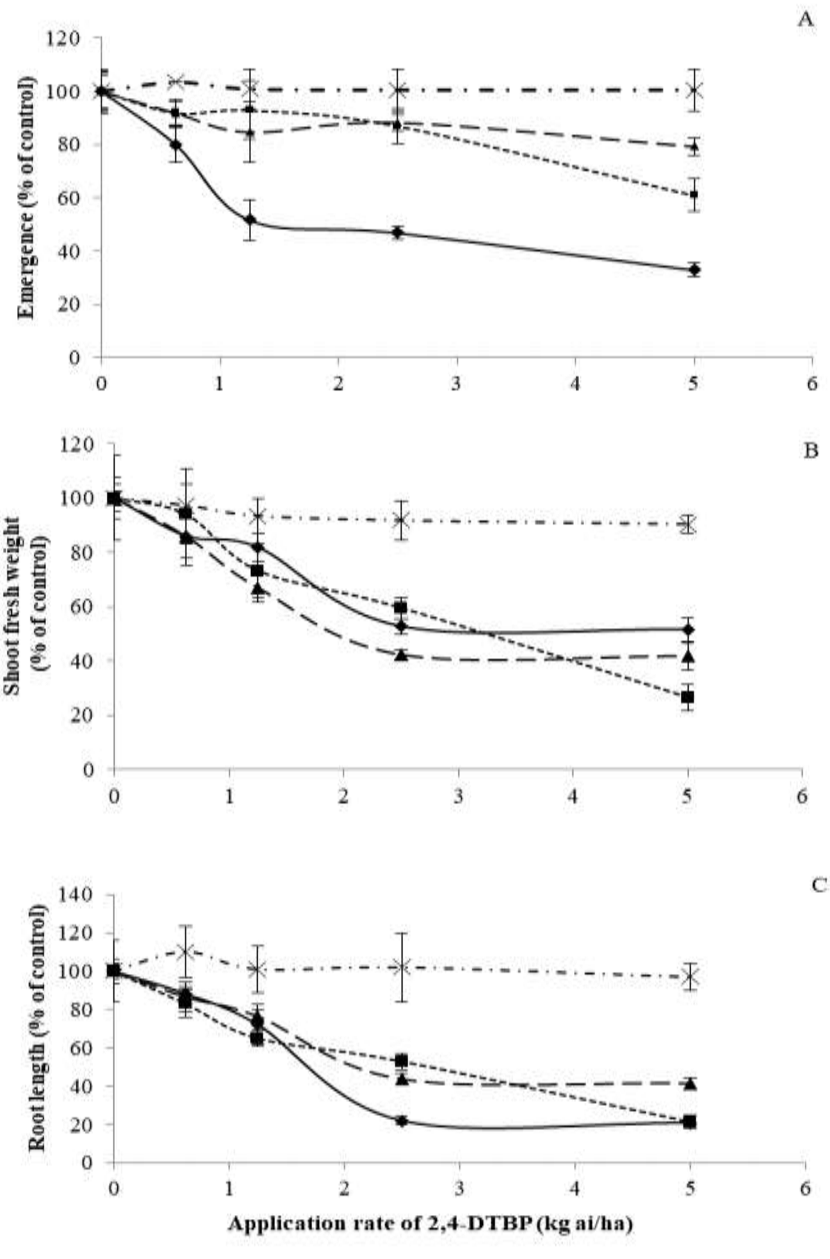

Figure 3. Pre-emergence application of 2,4-di-tert-butylphenol (2.4-DTBP) on emergence (A), shoot fresh weight (B) and root length (C) of *Leptochloa chinensis* ( ), *Eleusine indica* ( ), *Oldenlandia verticillata* ( ) and *Asystasia gangetica* ( ) 21 days after treatment. Vertical bar represents standard deviation (SD) of the mean.

In this study, the allelochemical 2,4-DTBP exhibited strong herbicidal activity as pre-emergence herbicide on *L. chinensis, E. indica* and *O. verticillata,* when applied onto the soil surface (Figure 2A). At 2.5 kg ai/ha 2,4-DTBP the seedling emergence of *L. chinensis* was reduced by 53 %. Likewise, the applied dibutyl phthalate (a natural compound extracted from *Chrysopogon serrulatus* Trin) as pre emergence at 2.4 kg ai/ha reduced he emergence of *L. chinensis* by 50 % (8). This result suggests that 2,4-DTBP exhibited similar promising pre-emergence herbicidal activity as dibutyl phthalate on inhibition of *L. chinensis* emergence.

The susceptibility of target species to phytotoxic substances depends on the biochemical and physiological characteristics of each weed species (21). In the present study, the emergence, shoot and root growths of *A. gangetica* were not affected by 2,4-DTBP treatment regardless of any application rates. Samedani *et al*. (33) reported that the application of litter leachate of *Axonopus compressus* Sw. P. Beauv. in soil, stimulated the seedling growth of *A. gangetica*. This indicated that *A. gangetica* was not affected by different litter leachate concentrations, although the litters contained many allelopathic compounds. The response of *A. gangetica* to 2,4-DTBP differs from other 3-weeds bioassay species, it might be due to evolutionary differences in tolerance of allelopathic compounds among the target species (17,30).

CROP TOLERANCE TEST

The degree of susceptibility of test crop plants (*B. rapa*, *Z. mays* and *O. sativa*) to 2, 4-DTBP treatment was evaluated at different growth stages. There were no significant interactions between the 2,4-DTBP doses and growth stage of test crop species in shoot fresh weight. Hence, data were pooled and the main effects were presented. Average across the 2,4-DTBP rate and growth stage, shoot fresh weights of all tested crop plants were from 97 to 104 % of non-treated control, suggesting that shoot growth of crops was not affected by 2,4-DTBP treatment (Figure 4).

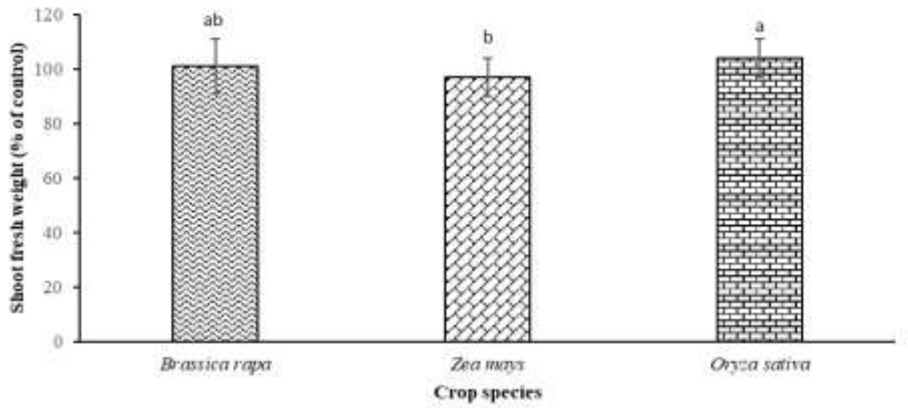

Figure 4. Main effect of crop species in response to 2,4-di-tert-butylphenol application on crop shoot fresh weight 14 days after treatment. Vertical bar represents standard deviation (SD) of the mean. Main effect means followed by the similar letter has no significant difference at $p \leq 0.05$ after determined by Tukey test.

Average across 2,4-DTBP rates, the root lengths at 0 day after sowing/transplant (DAS) were 86 % for *O. sativa* and 90 % for *B. rapa* than non-treated control. It was observed that starting at 4 DAS, the root lengths of these two crop plants were less affected by the 2,4-DTBP treatment and the root length of *Z. mays* was 96 % of non-treated control. It is interesting to note that root length of *Z. mays* was not affected at any growth stage (Figure 5).

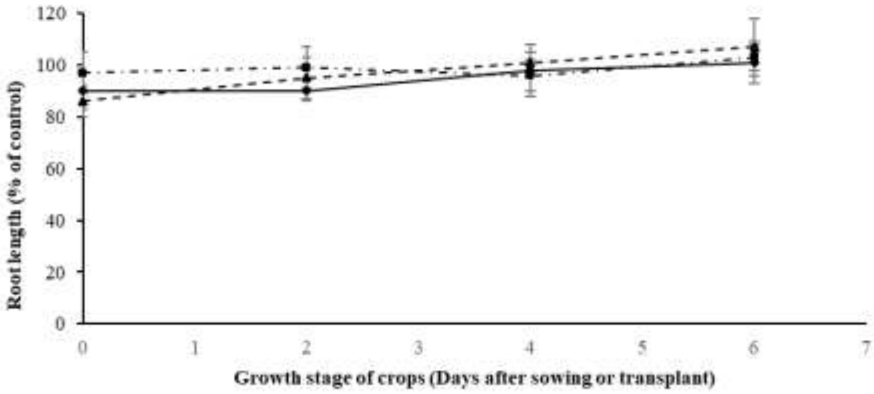

Figure 5. Inhibitory effect of 2,4-di-tert-butylphenol on root growth of *Brassica rapa* ( ), *Zea mays* ( ) and *Oryza sativa* ( ) in different growth stages at application, 14 days after treatment. Vertical bar represents standard deviation (SD) of the mean. Seeds of *Z. mays* and *O. sativa* were sown while *B. rapa* seedlings were transplanted.

Generally, all test crop plants were tolerant or slightly inhibited by 2,4-DTBP depending on the bioassay species. Shoot growths of *Z. mays*, *B. rapa* and anaerobic *O. sativa* were healthy after 2,4-DTBP treatment irrespective of any application rates, when applied as pre or post emergence (Figure 4). However, the root growth of anaerobic *O. sativa* and *B. rapa* were slightly inhibited (10-15 % of non-treated control), when averaged across 2,4-DTBP application rates, 14 days after treatment (Figure 5). Still, *B. rapa* was more tolerant to 2,4-DTBP treatment than aerobic *O. sativa* where after application of 2,4-DTBP, the root growth was reduced by 30 % (8).

The microbial degradation of phenol derivatives (4-i-propylphenol, 4-n-butylphenol, 4-sec-butylphenol, 4-t-butylphenol and 4-t-octylphenol) in soil took 224 days in anaerobic incubation in seven Japanese paddy soil, whereas these phenol derivatives were degraded within 16 days of incubation in aerobic condition (34). Based on above findings, it is hypothesized that 2,4-DTBP also phenolic compound, is most likely to persist longer under anaerobic conditions than under aerobic conditions, thereby causing greater phytotoxicity under anaerobic conditions. Nevertheless, root growth of anaerobic *O. sativa* tested was less affected by 2,4-DTBP treatment than aerobic *O. sativa* (8), suggesting that the anaerobic *O. sativa* is more tolerant to 2,4-DTBP treatment than aerobic *O. sativa*.

High tolerance level of these test crops to 2,4-DTBP may be due to their large seed sizes as compared to small seeds of weeds (*L. chinensis*, *E. indica* and *O. verticillata*) The allelochemical 2,4-DTBP at 5 kg ai/ha inhibited the seedling growths of these weed species by 50 % (Figure 3), whereas, the shoot growths of crop species were not inhibited (Figure 5). Variations among the species in seed mass may lead to the differential susceptibility to allelochemical. Previous studies showed that small seeded species were more susceptible to allelochemicals due to greater surface to volume ratio and thus its exposure per unit mass to allelopathic substances in the soil was also greater (3,29). Leishman *et al*. (22) claimed that large seeded species like *Z. mays* generally had greater reserves to support the seedlings respiration longer during stress-induced carbon deficiency. Besides, Liebman and Sundberg (24) reported that *Phaseolus vulgaris* L. seeds with large reserves were also able to tolerate and detoxify the allelopathic agents.

Contrarily to our present study, recent studies on sorgoleone (37) and coumarin (28) showed that crop plants were sensitive to the allelochemicals. Application of sorgoleone at 0.4 kg ai/ha as post emergence stage on 3 weeks old plants of *B. rapa* reduced their shoot fresh weight (19 %), whereas, the shoot growth of *Z. mays* was slightly inhibited (8 and 11 %) for pre and post application of 0.4 kg ai/ha sorgolene, respectively (37). Nazemi *et al*. (27) reported that at coumarin at 1.9 kg ai/ha when applied as pre-emergence reduced (20 %) the shoot fresh weight of *Z. mays.*

CONCLUSIONS

The allelochemical 2,4-DTBP has poor post emergence herbicidal activity in the soil. However, the pre emergence application of 2,4-DTBP at 2.5 kg ai/ha controlled the test weed species (*L. chinensis*, *O. verticillata* and *E. indica*). The 2,4-DTBP effective rate of 2.5 kg/ha was comparable to the recommended rates (2-4 kg ai/ha) of commercial synthetic herbicides such as thiobencarb and atrazine. The tolerance of test crop plants to 2,4-DTBP depended on the species and growth stage. *Z. mays* was tolerant to 2,4-DTBP treatment regardless of any growth stages, even at dose of 5 kg ai/ha. Meanwhile, *O. sativa* and *B. rapa* were not injured by 2,4-DTBP when applied 4 days after transplanting, respectively. These results imply that the allelochemical 2,4-DTBP has the potential to be developed as natural pre-emergence herbicide for weed control in vegetable farms, rice and corn fields.

REFERENCES

1. Ajayi, G., Olagunju, J. A., Ademuyiwa, O. and Martins, O.C. (2011). Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of *Plumbago zeylanica*, Linn. *Journal of Medicinal Plants Research* **5:** 1756-1761.
2. Bandyopadhyay, S., Chowdhury, R. and Bhattacharjee, C. (2014). Production of biosurfactants through bio-desulfurization of spent engine oil - an experimental study. *Green Chemistry Letters and Reviews* **7(3):** 288-295.
3. Chase, W.R., Nair, M.G. and Putnam, A.R. (1991). 2,2'-oxo-1,1'-azobenzene: selective toxicity of rye (*Secale cereal* L.) allelochemicals to weed and crop species. *Journal of Chemical Ecology* **17:** 9-19.
4. Chon, S.U., Jang, H.G., Kim, D.K., Kim, Y.M., Boo, H.O., and Kim, Y.J. (2005). Allelopathic potential in lettuce (*Lactuca sativa* L.) plants. *Scientia Horticulturae* **106:** 309-317.
5. Chuah, T.S., Md Zain, N. and Ismail, S. (2014b). Phytotoxic effects of the extracts and compounds isolated from Napiergrass (*Pennisetum purpereum*) on Chinese Sprangletop (*Leptochloa chinensis*) germination and seedling growth in aerobic rice systems. *Weed Science* **62:** 457-467.

6. Chuah, T.S., Noor-Zalila, M.R., Cha, T.S. and Ismail, B.S. (2005). Paraquat and glyphosate resistance in woody borreria (*Oldenlandia verticillata*) growing at oil palm plantations in Terengganu, Malaysia. *Malaysian Applied Biology* **34(2):** 43-49.
7. Chuah, T.S., Norhafizah, M.Z. and Ismail, B.S. (2015). Evaluation of the biochemical and physiological activity of the natural compound, 2,4-ditert-butylphenol on weeds. *Crop and Pasture Science* **66:** 214-223.
8. Chuah, T.S., Oh, H.Y., Habsah, M., Norhafizah, M.Z. and Ismail, B.S. (2014a). Potential of crude extract and isolated compounds from golden beard grass (*Chrysopogon serrulatus*) for control of sprangletop (*Leptochloa chinensis*) in aerobic rice systems. *Crop and Pasture Science*. **65:** 461-469.
9. Chuah, T.S., Tiun, S.M. and Ismail, B.S. (2011). Allelopathic potential of crops on germination and growth of goosegrass (*Eleusine indica* L. Gaertn) weed. *Allelopathy Journal* **27(1):** 33-42.
10. Chuah, T.S., Zain, N.M., Naimah, A.H. and Ismail, B.S. (2016). Phytotoxic activity of the allelochemical, 2,4-di-tert-butylphenol on two selected weed species. *Sains Malaysiana* **45:** 963-967.
11. Dayan, F.E., Howell, J.L. and Weidenhamer, J.D. (2009). Dynamic root exudation of sorgoleone and in plants its mechanism of action. *Journal of Experimental Botany* **1(11):** 2107-2117.
12. EPA, (2001). *Alkalphenols Category* Section 1: *Development of Categories and Test Plans*. US Environment Protection Agency, New York.
13. Halim, N.A., Ma, N.L., L.M., Sahid, I. and Chuah. T.S. (2018). Effects of 2,4-di-tert-butylphenol and selected lipid peroxidase herbicides on quantum yield and membrane integrity of weedy plants under dark and light conditions. *Sains Malaysiana* **47(2):** 261-268.
14. Halim, N.A., Razak, S.B.A. Simbak, N. and Seng, C.T. (2017). 2,4-di-tert-butylphenol-induced leaf physiological and ultrastructural changes in chloroplasts of weedy plants. *South African Journal of Botany* **112:** 89-94.
15. Heisey, R.M. (1996). Identification of an allelopathic compound from *Ailanthus altissima* (Simaroubaceae) and characterization of its herbicidal activity. *American Journal of Botany* **83:** 192-200.
16. Huang, X.J., Li, Y.C., Jiang, P., Zhang, X., Zhang, X.Y., Tan, P.Y. and Tian, W. (2017). Identification of chrysanthemum root exudates and allelopathic effects of three plants. *Hubei Agricultural Sciences* **56:** 1061-1071. (Chinese).
17. Hunter M.E. and Menges, E.S. (2002). Allelopathic effects and root distribution of *Ceratiola ericoides* (Empetraceae) on seven rosemary scrub species. *American Journal of Botany* **89:** 1113-1118.
18. Inderjit, B.D. and Raheswari, M.S. (2010). Interaction of 8-hydroxyquinolline with soil environment mediates its ecological function. *Plos One* **5(9):** e2852.
19. John, J., Shirmila, J., Sarada, S. and Anu, S. (2010). Role of Allelopathy in vegetables crops production. *Allelopathy Journal* **25:** 275-312.
20. Kang, X.L., Liu, X. and Luo, J.R. (2014). Analysis of fat-soluble constituents in pine cone of *Pinus yunnanensis* from various habitats by GC-MS. *Chinese Journal of Experimental Traditional Medical Formulae* **20:** 106-109.
21. Kobayashi, K. (2004). Factors affecting phytotoxic activity of allelochemicals in soil. *Weed Biology and Management* **4:** 1-7.
22. Leishman, M.R., Wright, I.J., Moles, A.T. and Westoby, M. (2000). The evolutionary ecology of seed size. In *Seeds: The Ecology of Regeneration in Plant Communities* (Ed., M. Fenner), pp.31-57. CAB International, Sydney.
23. Li, Z.R., Amist, N. and Bai L.Y. (2019). Allelopathy in sustainable weeds management. *Allelopathy Journal* **48(2):** 109-138.
24. Liebman, M. and Sundberg, D.N. (2006). Seed mass affects the susceptibility of weed and crop species to phytotoxins extracted from Red clover shoots. *Weed Science* **54:** 340-345.
25. Lotina-Hennsen, B., King-Díaz, B. and Pereda-Miranda, R. (2013). Tricolorin A as a natural herbicide. *Molecules* **18:** 778-788.
26. Luo, J.R., Li, D.M., Zhang, Z. and Liu, G.M. (2011). GC-MS analysis on the constituents of n-hexane extracts from the pinecone of *Pinus kesiya* var. *langbianensis. Journal of Anhui Agricultural Sciences* **39:** 18647-18648. (Chinese).
27. Muthusamy, K., Gopalakrishnan, S., Ravi, T. and Sivachidambaram, P. (2008). Biosurfactants: Properties, commercial production and application. *Current Science* **94:** 736-747.
28. Nazemi, A.H., Asadi, G.A. and Ghorbani, R. (2015). Herbicidal activity of coumarin when applied as a pre-plant incorporated into soil. *Notulae Scientia Biologicae* **7(2):** 239-243.
29. Putnam, A.R. and DeFrank, J. (1983). Use of phytotoxic plant residues for selective weed control. *Crop Protection* **2:** 173-181.

30. Qasem, J.R. (2001). Allelopathic potential of white top and Syrian sage on vegetable crops. *Agronomy Journal* **93:** 64-71.
31. Rahman, M.M., Ismail, S. and Sofian-Azirun, M. (2011). Identification of resistant biotypes of *Leptochloa chinensis* in rice field and their control with herbicides. *African Journal of Biotechnology* **10(15):** 2904-2914.
32. Rana, V.S. and Blazquez, M.A. (2007). Chemical constituents of *Gynura cusimbua* aerial parts. *Journal of Essential Oil Research* **19:** 21-22.
33. Samedani, B., Juraimi, A.S., Anwar, M.P., Rafii, M.Y., Awadz, S.A.S. and Anuar, A.R. (2013). Phytotoxic effects of *Pueraria javanica* litter on growth of weeds *Asystasia gangetica* and *Pennisetum polystachion*. *Allelopathy Journal* **32(2):** 191-202.
34. Shibata, A., Inoue, Y. and Katayama, A. (2006). Aerobic and anaerobic biodegradation of phenol derivatives in various paddy soils. *Science of the Total Environment* **367:** 979-987.
35. Singh, H.P., Batish, D.R., Kaur, S., Ramezani, H. and Kohli, R.K. (2002). Comparative phytotoxicity of four monoterpenes against *Cassia occidentalis*. *Annals of Applied Biology* **141:** 111-116.
36. Tian, K. and Wang, X.Y. (2018). GC-MS analysis of water extracts of pine needles of *Pinus tabulaeformis*. *Tianjin Agriculture for Science and Technology* **2018**: 1-10. (Chinese).
37. Uddin, M.R., Park, S.U., Dayan, F.E. and Jong, Y.P. (2013). Herbicidal activity of formulated sorgoleone, a natural product of sorghum root exudate. *Pest Management Science* **70:** 252-257.
38. Weir, T.F., Park, S.W. and Vivanco, J.M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current Opinion in Plant Biology* **7:** 472-479.
39. Xuan, T.D., Toyama, T., Fukuta, M., Khanh, T.D. and Tawata, S. (2009). Chemical interaction in the invasiveness of cogongrass (*Imperata cylindrica* (L.) Beauv.). *Journal of Agricultural Food Chemistry* **57:** 9448-9453.
40. Yang, C.M., Lee, C.N. and Chou, C.H. (2002). Effects of three allelopathic phenolics on chlorophyll accumulation of rice (*Oryza sativa*) seedlings: I. Inhibition of supply-orientation. *Botanical Bulletin of Academia Sinica* **43:** 299-304.