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Phytotoxic Activity of the Allelochemical, 2,4-Di-Tert-Butylphenol on Two Selected Weed Species

(Aktiviti Fitotoksik Alelokimia, 2,4-Di-Tert-Butilfenol terhadap Dua Spesies Rumpai Terpilih)

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ABSTRACT

The allelochemical 2,4-di-tert-butylphenol (2,4-DTBP) is one of the natural compounds present in medicinal plants. This compound has been reported to possess herbicidal properties. However, its effect on weed growth parameters is unknown for it to be utilized in weed management. Hence, the herbicidal potential of the allelochemical 2,4-DTBP on the root and leaf tissues of the grassy weed, Leptochloa chinensis *(L.) Nees and the broadleaf weed,* Hedyotis verticillata *(L.) Lam was investigated. After 2,4-DTBP treatment, both bioassay species had abnormal and much shorter root hairs compared to those of untreated plants. The roots of* H. verticillata *were severely damaged with the root nodes turned brown. The phytotoxic effect of 2,4-DTBP on* L. chinensis *and* H. verticillata *became apparent at seven days and 14 days after treatment with symptoms of lamina wilting and necrosis, respectively. These results demonstrated that 2,4-DTBP could be used as a natural herbicide for the control of* L. chinensis *and* H. verticillata.

Keywords: Allelochemical; Hedyotis verticillata*;* Leptochloa chinensis*; natural herbicides; weed control*

ABSTRAK

Alelokimia 2,4-di-tert-butilfenol (2,4-DTBP) adalah salah satu sebatian semula jadi yang terdapat dalam tumbuhan perubatan. Sebatian ini dilaporkan mempunyai ciri-ciri fitotoksik. Walau bagaimanapun, kesan 2,4-DTBP ke atas parameter pertumbuhan rumpai tidak diketahui untuk kegunaannya dalam pengurusan rumpai. Oleh itu, potensi fitotoksik daripada alelokimia 2,4-DTBP pada akar dan tisu daun rumpai berdaun tirus, Leptochloa chinensis *(L.) Nees dan rumpai berdaun lebar,* Hedyotis verticillata *(L.) Lam telah dikaji. Selepas rawatan 2,4-DTBP, kedua-dua spesies bioasai mempunyai rerambut akar yang tidak normal dan lebih pendek berbanding dengan tumbuhan yang tidak dirawat. Akar* H. verticillata *mengalami kerosakan teruk dengan nod akar bertukar coklat. Kesan fitotoksik 2,4-DTBP pada* L. chinensis *dan* H. verticillata *menjadi jelas pada hari ketujuh dan ke-14 selepas rawatan dengan simptom layu dan nekrosis pada lamina daun masing-masing. Hasil kajian ini menunjukkan bahawa 2,4-DTBP boleh digunakan sebagai racun herba semula jadi bagi mengawal* L. chinensis *dan* H. verticillata.

Kata kunci: Alelokimia; Hedyotis verticillata*; kawalan rumpai;* Leptochloa chinensis*; racun semula jadi*

INTRODUCTION

The negative impact of commercial herbicides with regard to the environment and human health makes it necessary to diversify weed management options (Jurewicz & Hanke 2008). Plants produce hundreds of secondary metabolites and some of these compounds show allelopathic activity such as growth inhibitory effects on other plants (Macías et al. 2007). Thus, the use of these allelochemicals is one strategy to reduce dependency on commercial herbicides in practical weed control programs (Farooq et al. 2011). In addition, these natural compounds are considered to be more environmentally benign than most of the synthetic herbicides (Macías et al. 2007). Several recent studies have indicated the potential use of compounds isolated from weeds such as jungle rice (*Echinochloa colona* (L.) Link) (Gomaa & AbdElgawad 2012), congongrass (*Imperata cylindrica* [L.] P. Beauv) (Cerdeira et al. 2012), croftonweed (*Ageratina adenophora* (Spreng.) King & H. Rob) (Zhang et al. 2012) and wild sage (*Lantana camara* L.) (Hussain et al. 2011), as alternatives to the use of synthetic herbicides. Furthermore, a number of allelochemicals are already commercially available or in the process of being manufactured on a large-scale. For example, mesotrione (trade name Callisto) which was isolated from a natural compound from lemon bottle brush (*Callistemon citrinus* Splendens) has been developed as herbicide for selective weed control in corn (Bhownik & Zhang 2003).

2,4-di-tert-butylphenol (2,4-DTBP) is one of the natural compound present in medicinal plants like *Gynura cusimbua* (D. Don) S. Moore (Rana & Blazquez 2007), *Pereskia bleo* (Kunth) de Candolle (Sri Nurestri et al. 2009), *Heliotropium indicum* L. (Oluwatoyin et al. 2011) and *Plumbago zeylanica* L. (Ajayi et al. 2011). This compound has been reported to have medical properties such as antioxidant (Choi & Lee 2009; Kadoma et al. 2009), anticancer (Sri Nurestri et al. 2009), antifungal (Zhou et al. 2011) and antibacterial activities (Abdullah et al. 2011). In food processing, this compound has been proposed to prevent browning in fresh apple juices (Suh et al. 2011). Zhang et al. (2011) have identified 2,4-DTBP in rhizosphere soil extracts of hops (*Humulus lupulus* L.). The results suggested that autotoxicity caused by this compound in rhizosphere soil could be one of the reasons for quality degradation in hops. Besides, 2,4-DTBP extracted from the rhizome of cogongrass has been found to have allelopathic effects on the germination and seedling growth of weedy plants under soil-less conditions. For instance, 2,4-DTBP at 100 µg mL^{-1} completely inhibited the germination of *Imperata cylindrica* (L.) P. Beauv and showed 78-95% inhibition on the root and shoot growth of beggar's tick (*Bidens pilosa* L.), Leucaena (*Leucaena leucocephala* (Lam.) de Wit) and barnyardgrass (*Echinochloa crus-galli* (L.) Beauv) (Xuan et al. 2009). Recently, Chuah et al. (2014) have identified 2,4-DTBP in culm and leaf extracts of napier grass (*Pennisetum purpureum* Schumach). They found that 2,4-DTBP exhibited potent herbicidal activity where it completely inhibited the germination of *Leptochloa chinensis* at the concentration of 500 µg mL^{-1} and completely prevented root growth of *L. chinensis* at an application rate of as low as 0.60 kg ai ha^{-1} under soil conditions. Further study by Chuah et al. (2015) showed that this compound acts by inducing oxidative stress through the generation of reactive oxygen species, which causes lipid peroxidation and membrane damage in root tissues and chloroplast in leaf tissues. However, not much is known about its effect on weed growth attributes for it to be utilized in weed management. Hence, the present study was conducted to examine phytotoxicity of 2,4-DTBP on root and leaf tissues of two selected weed species.

Materials and Methods

PLANT MATERIALS

Seeds of two bioassay species used in the study, namely, *Leptochloa chinensis* and *Hedyotis verticillata* were collected from rice fields at Pengkalan Maras, Kuala Terengganu (coordinates: 5°26'13.8"N and 103°3'33.4"E) and the oil palm plantation of Espek RISDA Gerdong, Kuala Berang (coordinates: 5°13'0"N and 102°51'0"E), respectively. Both the bioassay species were propagated in a glasshouse.

RAISING OF THE SEEDLINGS

Seeds of *L. chinensis* and *H. verticillata* were sown in seedling trays (40-by-30-by-5 cm; two seeds per hole) filled with commercial potting mixture. All trays were placed in a glasshouse with 12 h photoperiods and photosynthetic photon flux density (PPFD) 800±200 µE m^{-2}s^{-1}, temperatures ranging from 20 to 35°C and relative humidity of 70-80%. The seedlings were watered daily with tap water and raised until they reached the six-leaf stage (5-week old plant).

PLANT GROWTH ATTRIBUTES

Seedlings of *L. chinensis* and *H. verticillata* at six-leaf stage were transferred into glass vials (2 cm diameter × 7 cm height) filled with 1/8-strength Hoagland nutrient solution at pH 6.0±0.2 and electrical conductivity of 1.2 ms/cm. The vials were placed in a controlled growth room with a L/D regime of 12/12 h, temperature range of 30/20°C, a photon flux density of 140-160 µmol m^{-2} s^{-1} and relative humidity of 78-80%. *L. chinensis* and *H. verticillata* seedlings were allowed to acclimatize for two days in the Hoagland nutrient solution before the treatments were applied. High grade 2,4-DTBP (99% purity; purchased from Sigma Chemical Co., Kuala Lumpur, Malaysia), was dissolved in 2% dimethyl sulfoxide (DMSO) and added to the nutrient solution to concentrations of 50 µg mL^{-1} for *L. chinensis* and 200 µg mL^{-1} for *H. verticillata*. For the non-treated bioassay species in Hoagland nutrient, an addition of 2% DMSO was added as control treatment. The solution in the glass vial was maintained by adding 1/8-strength Hoagland nutrient solution at 24 h intervals. Treated and non-treated *L. chinensis* and *H. verticillata* seedlings were harvested at seven and 14 days after treatment, respectively. Leaves and roots of bioassay species were observed under the Olympus Stereo Microscope (Model SZX16) fitted with a digital camera (DP21-Sal Standalone Connection Kit, Japan).

Results and Discussion

EFFECT OF 2,4-DTBP ON WEED GROWTH ATTRIBUTES

The phenolic compound of 2,4-DTBP evaluated for its phytotoxicity on two weed species exhibited a similar pattern on growth inhibition at seven or 14 days after treatment. The bioassay species grown in 50 or 200 µg mL^{-1} 2,4-DTBP were substantially smaller compared to the untreated plants, with symptoms of leaf blade wilting and necrosis (Figure 1) as well as stunted root growth with abnormal root hairs (Figure 2). It was found that the toxic effect of 2,4-DTBP was manifested in *H. verticillata* roots by brown colouring of root nodes (Figure 3). This suggested that 2,4-DTBP was most likely translocated to the aboveground tissues causing severe chlorosis and necrosis of the leaf blades, thereby leading to retardation in the growth of the weed. This finding was in agreement with the finding of the study on the phytotoxic effects of BOA treatment on lettuce plants (*Lactuca sativa* L.) (Sanchez-Moreiras & Reigosa 2005). The test species had shorter and typically browning roots without hairs after treatment with 135 µg mL^{-1} BOA, besides the decrease in leaf biomass. Yang et al. (2002) reported that phenolics such as ferulic and p-coumaric acids at 100 µg mL^{-1} not only caused growth retardation in rice (*Oryza sativa* L.) seedlings, but also leaf dehydration, leaf shrinkage and a decrease in leaf width one week after treatment. In another study, Nimbal et al. (1996) showed that sorgoleone treatment at 358 µg mL^{-1} caused chlorosis in sensitive leaf tissues of large crabgrass

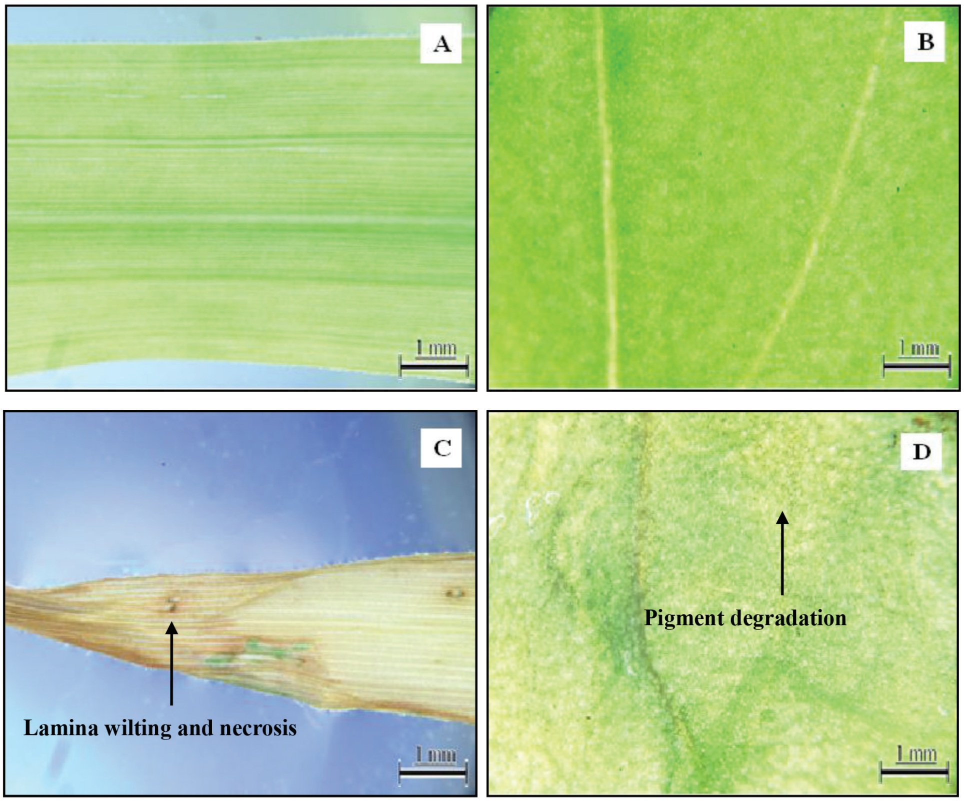

FIGURE 1. Normal lamina morphology of untreated *L. chinensis* (A) and *H. verticillata* (B) seedlings and lamina injuries in *L. chinensis* (C) and *H. verticillata* (D) seedlings treated at 50 µg mL^{-1} and 200 µg mL^{-1} 2,4-DTBP, respectively

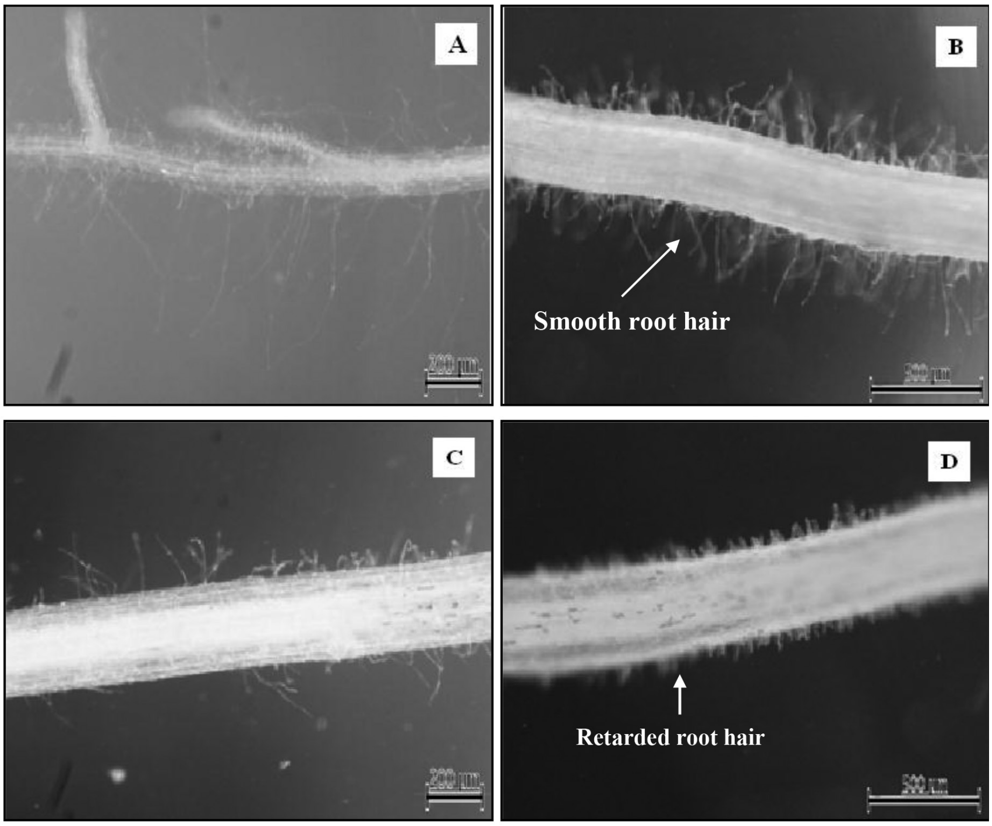

FIGURE 2. Healthy root of untreated *L. chinensis* (A) and *H. verticillata* (B) seedlings and root injuries in treated *L. chinensis* (C) and *H. verticillata* (D) seedlings treated at 50 µg mL^{-1} and 200 µg mL^{-1} 2,4-DTBP, respectively

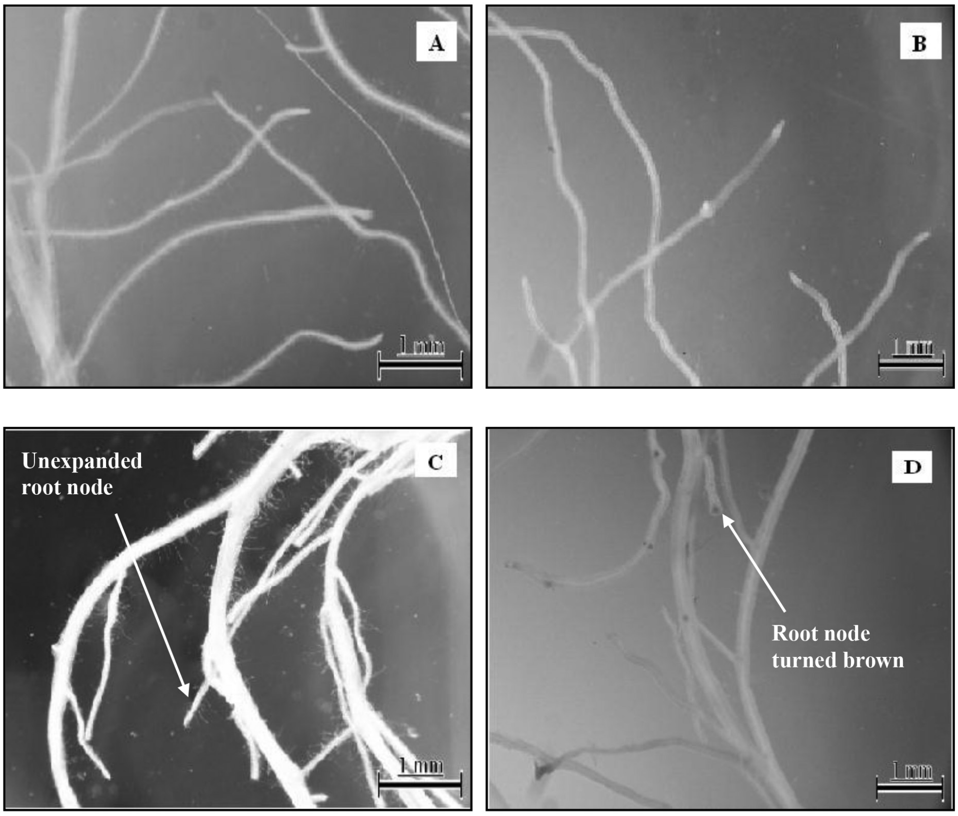

FIGURE 3. Normal root node of untreated *L. chinensis* (A) and *H. verticillata* (B) seedlings and abnormal root node of *L. chinensis* (C) and *H. verticillata* (D) seedlings treated at 50 µg mL^{-1} and 200 µg mL^{-1} 2,4-DTBP, respectively

(*Digitaria sanguinalis* (L.) Scop). The effect of sorgoleone on tissue chlorosis is an indication that sorgoleone may inhibit chlorophyll biosynthesis. These results are similar to those of a recent study conducted by Uddin et al. (2012) that burning and growth inhibition were observed 2-3 days after treatment with sorgoleone at 200 µg mL^{-1} in sensitive species (*Rumex japonicas* Houttuyn, *Galium spurium* L. and *Aeschynomene indica* L.) and significant stunting of growth was observed 14 days after treatment.

Although the EPA of USA has classified 2,4-DTBP as a pollutant which is toxic to aquatic organisms (EPA 2001) and mammals (Hirata-Koizumi et al. 2005), it has been demonstrated that 2,4-DTBP is biodegradable under aerobic conditions since this compound belongs to a group similar to the group of tertiary butylphenols (Shibata et al. 2006). Therefore, 2,4-DTBP has good potential to be developed as a natural herbicide for weed management programmes.

CONCLUSION

Based on the results of this study, it can be concluded that 2,4-DTBP affects the seedling growth of the bioassay species. After 2,4-DTBP treatment, *L. chinensis* and *H. verticillata* had abnormal and much shorter root hairs compared to those of untreated plants. The phytotoxic effect of 2,4-DTBP on the bioassay species was evident at seven days and 14 days after treatment with symptoms of lamina wilting and necrosis, respectively. Further study is currently in progress to characterize the herbicidal activity of 2,4-DTBP for the development of natural herbicide.

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