Plant Omics Journal POJ

POJ 14(01):30-37 (2021) ISSN:1836-3644
doi: 10.21475/POJ.14.01.21.p3265

Pre-emergence herbicidal activity and persistence of 2,4-di-tertbutylphenol in relation to soil types

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Abstract

Although 2,4-di-tert-butylphenol (2,4-DTBP) has demonstrated strong phytotoxic effect on various weedy plants in previous findings, research on its pre-emergence herbicidal activity in the soil is still scanty. The aim of this study was to investigate the effects of two soil types on pre-emergence herbicidal activity and persistence of 2,4-DTBP. The bioassay was carried out in a growth chamber where goosegrass [*Eleusine indica* (L.) Gaertn.] seeds were sown in different rates of 2,4-DTBP in two soil series under sterilized and non-sterilized soil conditions. Bioassays of each treatment were conducted in four replicates and arranged in completely randomized design. 2,4-DTBP exhibited potent pre-emergence activity as a root inhibitor where it completely inhibited (100% inhibition) of the root growth of *E. indica* in sandy loam soil at an application rate of 6.14 kg ai/ha. 2,4-DTBP was rapidly detoxified in silt loam soil as a result of high microbial activity where it completely lost its phytotoxicity by giving 100% emergence within 10 weeks even it was applied at an application as high as 20.4 kg ai/ha. However, 2,4-DTBP remained highly phytotoxic in sandy loam soil where it reduced the root and shoot growth by 47 and 36%, respectively, throughout 10 weeks duration of the investigation. The presence of microbes in non-sterilized soil further suggest that soil microbes may modify the chemical structure of the 2,4-DTBP, which in turn decreased its toxicity. The high level of pre-emergence herbicidal activity in conjunction with its biodegradation in silt loam soil imply that 2,4-DTBP may have potential for development as a natural-soil applied herbicide.

Keywords: Natural soil-applied herbicide; Phytotoxic compound; Goosegrass; Microbes; Soil series
Abbreviations: 2,4-DTBP_2,4-di-tert-butylphenol.

Introduction

2,4-di-tert-butylphenol or also known as 2,4-bis (1,1-dimethylethyl)-phenol (2,4-DTBP) is a toxic chemical compound released by various groups of organisms to the environment. It is one of constituents that present in medicinal plants like *Chimonanthus* (Fan et al., 2017), *Perilla frutescens* (Huang and Li, 2018), and *Gentiana apiata* (Xu et al., 2019). Besides, 2,4-DTBP was also found in *Camelia sinensis,* the flowering plant family whose leaves and leaf buds are used to produce tea (Zhu et al., 2018). 2,4-DTBP identified from different plant and fungus was also reported to possess antioxidant (Choi et al., 2013), insecticidal (Rajasekharan et al., 2019) and antifungal activity (Dharni et al., 2014). According to Leila et al. (2019) and Aissaoui et al. (2019), 2,4-DTBP identified from Tunisian olive twig cultivars and *Bacillus licheniformis,* respectively has shown antiviral and antibacterial activity. It has been documented that 2,4-DTBP in violate or essential oils are prone to exhibit potent toxicity against almost all testing organisms (Zhao et al., 2020).

The phenol has a potential to be used as natural soil applied herbicide which is environmentally friendly for weed management (Chuah et al., 2014). At an application rate of 0.60 kg a.i./ha, 2,4-DTBP which isolated from culm and leaves of napier grass (*Pennisetum purpureum* Schum.) can completely inhibit the root growth of *Leptochloa chinensis* (L.) Nees in soil (Chuah et al., 2014). Later on, a study conducted by Chuah et al. (2016) found that 2,4-DTBP showed toxic effects on the root and leaf tissues of the grassy weed, *L. chinensis* and broadleaf weed, *Hedyotis verticillata* (L.) Lam after 7 to 14 days of treatment with symptoms of lamina wilting and necrosis, respectively; with both had abnormal and much shorter root hairs compared to those of untreated plants. This compound also was reported to reduce the

chlorophyll content, chlorophyll fluorescence, transpiration, and net photosynthetic rate (Chuah et al., 2015) besides altering the chloroplast ultrastructure in the leaf tissues of weeds (Halim et al., 2017). It was also demonstrated that 2,4-DTBP has potent light-independent herbicidal activity which reduced the photosynthetic rate and causing the loss of membrane integrity of *Oldenlandia verticillata* and *L. chinensis* (Halim et al., 2018). Recently, Norhafizah et al. (2020) reported that the soil bioassay of pre-emergence herbicidal activity of 2,4-DTBP had reduced the seedling growth of several weeds (*L. chinensis*, *Eleusine indica* and *O. verticillata*) by 50- 80% at 2.5 kg ai/ha. The toxicity of 2,4-DTBP isolated from rhizome and root exudates of *Imperata cylindrica* (L.) Beauv was also documented on several weed species including beggar ticks (*Bidens pilosa* L.), leucaena (*Leucaena leucocaphala* L. de Wit), and barnyardgrass (*Echinochloa crus-galli* (L.) Beauv) (Xuan et al., 2009). 2,4-DTBP has been identified in rhizosphere soil extracts of hops plants (*Humulus lupulus* L.) by Zhang et al. (2011). They found that one of the reasons of quality degradation in hops could be due to soil autotoxicity of this compound. The allelopathic activity of 2,4-DTBP is concentration dependent where low concentrations of this phenolic was shown to promote the growth of hop seedlings in soil, whereas a high concentration inhibited their growth. Liu et al. (2017) reported that soil sickness can be caused by 2,4-DTBP based on an experiment in the field of *Boehmeria nivea* (L.) Gaudich. They observed that 2,4-DTBP significantly affect the abundance of culturable bacteria, fungi, and actinomycetes of the rhizosphere soil. Meanwhile, other studies discovered that high concentration of 2,4-DTBP can prevent seed germination and seedling growth of chilli pepper besides inhibiting the growth of eggplants at more than 2 mmol/L and between 0.10–1.00 mmol/L, respectively (Jiang et al., 2013). In spite of these multitude of studies, however, dissention remain on how 2,4-DTBP decomposed in the soil. It is indispensable to investigate the abiotic (physical and chemical) and biotic (microbial) soil barriers in environment that can limit the chemical or phytotoxic compound concentrations available to cause injury to the plants (Ohno, 2001; Salhi et al. 2013). In order to inhibit plant or weed growth, allelochemical or secondary metabolites must accumulate and persist at certain phytotoxic levels in the rhizosphere soil (Jilani et al., 2008). Although 2,4-DTBP has been demonstrated strong phytotoxic effect on various plants and weeds in previous findings, information on its pre-emergence activity in the soil is still scanty. The phytotoxic effects of 2,4-DTBP on goosegrass [*Eleusine indica* (L.) Gaertn.] was determined in this study due to its invasiveness and high abundance in paddy fields, orchards, oil palm plantations and vegetables farms. Besides, goosegrass is a problematic weed that has evolved resistance to several groups of commercial synthetic herbicides worldwide (Dilipkumar et al., 2017; Heap, 2020). Thus, the present study was conducted in order to evaluate effect of soil types on pre-emergence herbicidal activity of 2,4-DTBP on goosegrass and its persistence level in the soil.

Results

Pre-emergence phytotoxicity tests

The phytotoxic activity of 2,4-DTBP on *E. indica* in non-sterilized Renggam and Rhu Tapai soil series are presented in Figure 1. It is observed that emergence of *E. indica* were inhibited by more than 80% at 6.14 kg ai/ha in Rhu Tapai soil series and this inhibition was concentration dependent (Figure 1A). Comparatively, 2,4-DTBP displayed a greater response of allelopathic stress against radicle elongation where it completely inhibited the root growth of the bioassay species in Rhu Tapai soil series at same concentration (Figure 1B). The shoot growth of *E. indica* was also reduced gradually in Rhu Tapai soil series as the application rate of 2,4-DTBP increased (Figure 1C). The emergence and root growth of *E. indica* in Renggam soil series were reduced less than that in Rhu Tapai soil series when the application rate of 2,4-DTBP increased. The root growth of the bioassay species was only completely inhibited at the highest application rate of 16.00 kg ai/ha in Renggam soil series (Figure 1B). Meanwhile, the results for soil physicochemical and microbial analyses of Rhu Tapai and Renggam series are summarized in Table 1. It is noted that Rhu Tapai series is categorized as sandy loam soil while Renggam series is characterized as silt loamy soil. The soil pH of Rhu Tapai series is acidic (5.4), while soil pH of Renggam series is close to neutral (6.1). Besides, the total bacterial counts in Renggam and Rhu Tapai series are 3.4 x 104 cfu/g and 1.41 x 103 cfu/g respectively. It is interesting to note that Renggam series has higher CEC value as compared to that of Rhu Tapai series. This high CEC value is likely to have led to less availability of 2, 4-DTBP for *E. indica* root uptake due to higher 2,4-DTBP absorption onto the soil. As a result, Renggam series requires relatively higher rates of 2,4-DTBP to give 50% inhibition of emergence or seedling growth as compared to that of Rhu Tapai series.

Microbial degradation of 2,4-DTBP in soil

The rate that causes 50% inhibition (ED_{50}) and microbial degradation factor of *E. indica* in relation to 2,4-DTBP in Renggam and Rhu Tapai soil series are presented in Table 2. The ED_{50} values of root length for *E. indica* treated with 2,4-DTBP under non-sterilized soil conditions were higher than those of under sterilized soil condition regardless of any soil series. Similar trend was also observed on seed emergence but the ED_{50} of emergence was not significant different between sterilized and non-sterilized Rhu Tapai soil series. It is observed that microbial degradation factor was markedly higher in Renggam soil series as compared to that in Rhu Tapai soil series. The results presented herein clearly show that phytotoxic effect of 2,4-DTBP to *E. indica* under sterilized soil were higher as compared to those of under non-sterilized soil, implying that the presence of microbes in non-sterilized soil can degrade 2,4-DTBP compound. Thus, higher application rate of 2,4-DTBP was required to give 50% inhibition of root growth for *E. indica* under non-sterilized soil condition as compared to those of under sterilized soil conditions (Table 2).

Persistence of 2,4-DTBP in soil

Effects of pre-emergence treatment of 2,4-DTBP on emergence, root and shoot growth of *E. indica* under Renggam and Rhu Tapai soil series throughout 10-week incubation period is shown in Figure 2. There was an interaction between soil type and incubation period ($p<0.05$). When applied at 20.4 kg ai/ha, 2,4-DTBP in Rhu Tapai series soil had completely inhibited the emergence, root and shoot growth of *E. indica* during two weeks of incubation. At 10^{th} week after incubation,

Table 1. Soil physico-chemicals and microbial analyses of Renggam and Rhu Tapai series.

Parameter	Renggam series	Rhu Tapai series
Soil texture	Silt loam	Sandy loam
Clay, %	2.4	3
Silt, %	71.7	26.8
Sand, %	25.8	70.2
Organic carbon, %	0.9	3.5
Cation exchange capacity, meq[a] $100g^{-1}$	3.7	1.3
pH	6.1	5.5
Total bacterial count, CFU g^{-1}	3.4×10^4	1.41×10^3

[a]Abbreviations: meq, milliequivalents; CFU, colony forming units.

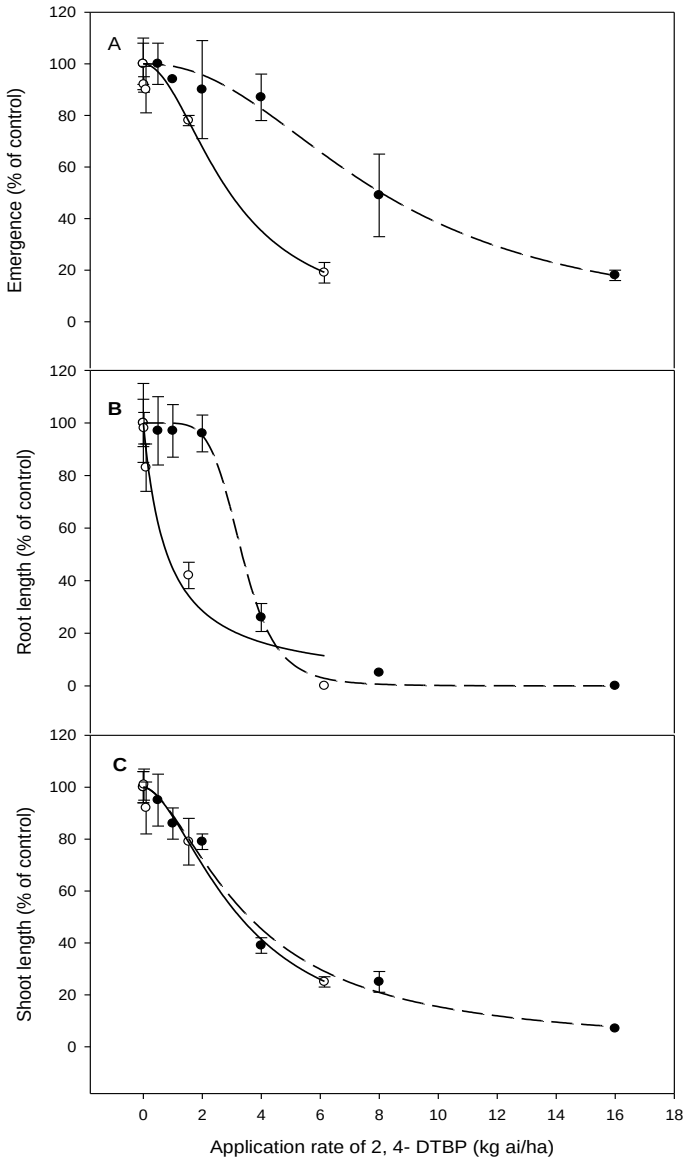

Fig 1. Effects of 2, 4-di-tert-butylphenol (2,4-DTBP) on emergence (A), root length (B) and shoot length (C) of Eleusine indica in non-sterilized Renggam (— — —) and Rhu Tapai (———) soil series. Vertical bars represent standard deviation (SD) of the mean.

Table 2. ED_{50} values and microbial degradation factor of Eleusine indica in relation to 2,4-DTBP in Renggam and Rhu Tapai soil series.

# ED_{50} (kg ai/ha)					^{x} Microbial degradation factor	
	Sterilized soil		Non-sterilized soil			
	Renggam series	Rhu Tapai series	Renggam series	Rhu Tapai series	Renggam series	Rhu Tapai series
Emergence	3.70 (0.38)	3.77 (0.15)	8.10 (0.56)	3.83 (0.20)	2.19	1.02
Root length	0.68 (0.04)	0.71 (0.16)	3.35 (0.14)	1.53 (0.13)	4.93	2.15

 # ED_{50} is the 2,4-DTBP application rate (kg ai/ha) required to reduce root length of Eleusine indica by 50%. The values in parentheses are the standard error of the mean. ^{x} Microbial degradation factor is calculated as ED_{50} of non-sterilized soil / ED_{50} of sterilized soil.

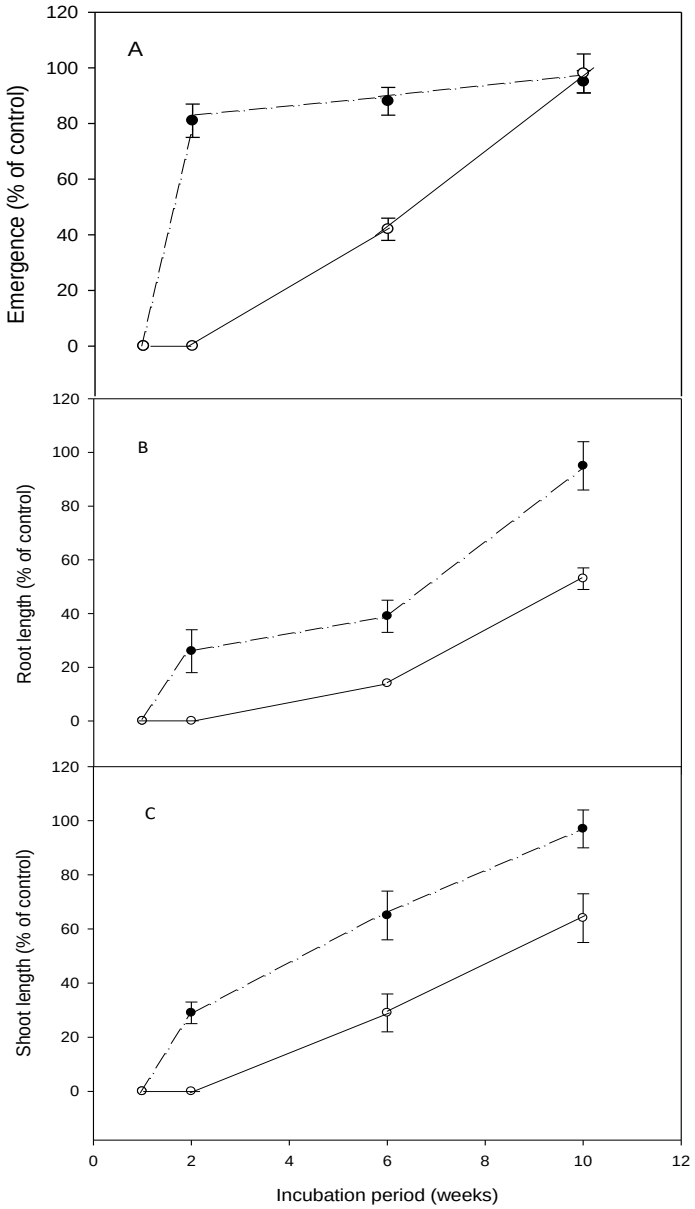

Fig 2. Effects of 2, 4-di-tert-butylphenol (2,4-DTBP) on emergence (A), root length (B) and shoot length (C) of Eleusine indica in Renggam (··—·—) and Rhu Tapai (———) soil series. Vertical bars represent standard deviation (SD) of the mean.

however, inhibition of 2,4-DTBP on emergence almost completely disappeared but this compound remained highly toxic to seedling growth of *E. indica* with root and shoot length being reduced by 47 and 36%, respectively. 2,4-DTBP in Renggam series soil completely inhibited the emergence, root and shoot growth of *E. indica* during one week of incubation. Nevertheless, it is found that toxicity of 2,4-DTBP to the bioassay species almost completely disappeared after 10 weeks of incubation. Overall, it is apparent that 2,4-DTBP was less persistence in Renggam soil series as compared to Rhu Tapai soil series. In addition, most of the *E. indica* seedlings in Renggam series soil remained alive and healthy but the emergence of *E. indica* was delayed and many seedlings that eventually did emerge were damaged and deformed because of strong inhibition on root and shoot growth in Rhu Tapai soil series after 10 weeks of incubation.

Discussion

Root growth of *E. indica* in both soil series was greatly inhibited by 2,4-DTBP as compared to other plant parameters like shoot growth and emergence. These findings imply that 2,4-DTBP compound is a strong root inhibitor rather than a shoot or germination inhibitor. Many factors must be considered before seriously pursuing the development of 2,4-DTBP as a natural pre-emergent herbicide. One of the most important factors is efficacy of 2,4-DTBP compared to commercial pre-emergence herbicides. The best weed control in yacon was achieved with pre-emergence herbicides of acetochlor, metribuzin and dimethanamid with an application rate of 2.50, 0.75, and 1.35 kg ai/ha, respectively. Seven weeks after application, these treatments had less than 1% weed cover and low weed numbers (Scheffer et al., 2002). Based on the present investigation, an application rate of 2,4-DTBP at 6.14 to 8.0 kg/ha in both series soil is found to be effective because it is able to cause 95 to 100% inhibition of root growth in *E. indica*. Nevertheless, this application rate is still much higher as compared to the rates of commercial herbicides. The high rate of 2,4-DTBP can be reduced by the addition of appropriate adjuvant to enhance its performance. Therefore, further study is needed in order to examine various types of adjuvants in improving 2,4-DTBP activity in soil.

In recent years, a number of bacteria in soil have been described as being capable of degrading aromatic compounds like phenol. For example, Shinoda et al. (2005) have isolated *Magnetospirillum* strains degrading tert-butylphenol from dump soils and paddy soils under nitrate-reducing conditions. Toyama et al. (2010) have reported that *Sphingobium fuliginis* Strain TIK-1 from *Phragmites australis* rhizosphere sediment have the ability to degrade 2,4-DTBP into 3,5-di-tert-butylcatechol which is less toxic. Likewise, it is believed that the 2,4-DTBP compound also could be degraded by the soil microbes in the present study, thereby reducing its herbicidal activity in soil.

The application rate of 20.4 kg ai/ha was examined in the persistence test using two different soil series based on a preliminary result of pre-emergence herbicidal test because this rate gave complete inhibition of emergence. Furthermore, this rate is in the range of concentration tested by Qu and Wang (2008) who examined effect of amendments with 2,4-DTBP on soil microbial biomass, activity, and community diversity. They found that low concentration of 2,4-DTBP compound at 0.02 – 0.20 mg/g in Luvic Phaeozems and black typical black soils could result in stimulation of microbial biomass, but inhibition was evident at a high concentration of 2.0 mg/g (Qu and Wang, 2008). In the present study, 20.4 kg ai/ha 2,4-DTBP is equivalent to 0.52 mg/g in soil. Hence, it is assumed that this application rate would not inhibit the microbial activity completely in soil.

Soil is capable of altering the bioavailability of allelochemicals (Tharayil et al., 2006) and these effects can be mediated not only by the soil physicochemical properties but also by the soil organic matter and organisms (Inderjit, 2001). The results of this study have demonstrated that the soil factors play important role in affecting the persistence of 2,4-DTBP in soils. It is apparent that 2,4-DTBP was rapidly neutralized in Renggam soil series as compared to its activity in Rhu Tapai soil series. This may be due to higher microbial activity in Renggam series (Table 2) that decreased the herbicidal activity of 2,4-DTBP by degrading or reducing its accumulation in the soil. As a result, 2,4-DTBP is less persistence in Renggam soil series as compared to Rhu Tapai soil series. In addition, it has been documented that the sorption of 4-nonylphenol increased with the increase in the organic matter in river sediments and soils (Düring et al., 2002). Hence, high persistence of 2,4-DTBP in Rhu Tapai series may be due to higher absorption onto organic carbon content, thereby retarding microbial degradation in the soil.

Herbicides that remain biologically active in soils after the treated crop is harvested may not be practically used. In fact, however, to be an effective herbicide 2,4-DTBP activity should be retained long enough to provide adequate weed control, but it must be degradable by soil microbes to become harmless metabolites before being applied in the soil again. A previous study has indicated that half-lives for aerobic microbial degradation of tertiary butylphenol detected in the paddy soils were ranged from 4 to 13 days (Shibata et al., 2006). These results suggest that 2,4-DTBP may have similar microbial degradation activity since this compound belongs to the similar group of tertiary butylphenol. According to Weston et al. (1999) sorgoleone from the root exudate of sorghum (*Sorghum bicolor* L.) was found to have persisted in soil for 8 weeks in small concentration and drastically degraded within 1 week. Furthermore, Xuan et al. (2003) found strong allelopathic inhibition effects of Kava (*Piper methysticum* L.) extracts on barnyard grass and monochoria growth (80%) 1 day after pre-emergence application; but at 9 days, the weed inhibition was reduced to 25%. Similarly, Xuan et al. (2005) reported that pre-emergence application for both alfalfa and kava extracts inhibited barnyard grass and monochoria weed growth by 80-100% for 10 days but after 20-25 days, the weed control was reduced to 50%. Thus, these results suggested that the phytotoxic level of allelochemicals in soil is largely controlled by soil factors such as adsorption, desorption and degradation. This in turn will minimize the phytotoxicity level that is required to cause injury for the plants.

Materials and Methods

Plant materials

Seeds of goosegrass [*Eleusine indica* (L.) Gaertn.] were purchased from seed supplier, Herbiseed, England. The outer

membranous bracts that enclose the *E. indica* seeds were scarified by using the sandpaper to accelerate germination.

Soil analysis

Soil samples of Renggam (silt loam) and Rhu Tapai (sandy loam) series were collected from Tepoh, Kuala Terengganu and Rhu Tapai, Marang, Terengganu, Malaysia, respectively. Soil samples were collected at 20 cm deep, air-dried, ground and sieved to pass a 2-mm screen. The soil pH was measured using a pH meter (Singh and Ratnasingham, 1977). Soil CEC was determined by ammonium acetate method at pH 7.00 (Chapman, 1965) while the Walkley-Black chromic acid wet oxidation method was used to determine of soil organic carbon (Mcleod, 1973). The soil textures were obtained by using textural triangle (Anderson and Ingram, 1993). Standard spread-plate dilution method was used to obtain the total bacterial counts (Seeley and Vandemark, 1981). Autoclaved and non-autoclaved soils were used in this study. The soil was autoclaved at 121°C and 103 kPa pressure for 30 minutes.

Chemicals

2,4-di-tert-butylphenol (99% purity) was purchased as high purity standards from Sigma Chemical Co.

Pre-emergence phytotoxicity tests

Under soil condition, a total of 50 seeds of *E. indica* were placed separately in 9-cm diameter Petri dish, each containing with 25 g of sieved and non-sterilized Renggam or Rhu Tapai soil series which moistened with 6.5 mL of 2,4-DTBP concentration solutions. Chloroform was used to dissolve 2,4-DTBP at rates of 0, 0.5, 1.0, 2.0, 4.0, 8.0 and 16 kg ai/ha and 0.024, 0.096, 0.384, 1.54, and 6.14 ai/ha in the respective soil series. Meanwhile for the control, 6.5 mL of chloroform was allowed to fully evaporate in Petri dish and then the samples were moistened with 6.5 mL of distilled water. The Petri dishes were kept in a growth chamber at $30/20^0$C with 12 hours photoperiod for 7 days. Seeds are considered germinated when attained a length of 1 mm. Evaporative water losses during incubation were determined and replenished with distilled water if necessary. At the end of the incubation period, the germinated seeds were recorded as a percentage of the total number of viable seeds used in each replication. The root length and shoot length of germinated seeds were measured and recorded. The data were expressed as percentage of control (Chuah et al., 2016).

Microbial degradation of 2,4-DTBP in soil

The role of soil microorganisms on degradation of 2,4-DTBP were examined in Renggam and Rhu Tapai soil series. Soil samples of each series were divided into two portions: one was autoclaved three successive times (103 kPa,120°C, 1 h) and the other remained unchanged according to the method of Xiao et al. (2020). Germination test of *E. indica* in sterilized and non-steriled Renggam and Rhu Tapai soil series were conducted by examining a series of 2,4-DTBP rates at 0, 0.25, 0.50, 1.00, 2.00, 4.00 and 8.00 kg ai/ha and 0, 0.024, 0.096, 0.384, 1.54, and 6.14 ai/ha in the respective soil series.

Persistence of 2,4-DTBP in soil

Degradation of the 2,4-DTBP in soil was examined in two soil types. Petri dishes were filled with 25 g of sieved Renggam or Rhu Tapai soil series. The soil in the dishes was moistened with 6.5 mL of 2,4-DTBP solution at a concentration of 20.4 kg ai/ha by dissolving it in chloroform. The treated dishes were incubated in the growth chamber at $30/20^0$C for 10 weeks. Evaporative water losses during incubation were determined by weighing every 14 days and replenished with distilled water. Dishes were removed from the growth chamber after incubation periods of 1, 2, 6 and 10 weeks. A total of 50 seeds of *E. indica* were sown in each Petri dish and incubated in the growth chamber again. After 7 days of sowing, the radicle length and shoot length of germinated seeds were measured and recorded. The data were expressed as percentage of their respective controls.

Experimental design and Statistical analysis

Bioassays of each treatment were conducted in four replicates and arranged in completely randomized design. All the percentage data were fitted to a logistic regression model, as follows Kuk et al. (2002) except the data from persistence test of 2,4-DTBP in soil:

$Y = d/ (1 + [x/x0]^{b})$

where Y is percentage of emergence/root length/shoot length, d is the coefficients corresponding to the upper asymptotes, b is the slope of the line, x0 is 2,4-DTBP concentration required to inhibit the emergence/root length/shoot length by 50% relative to untreated seedlings and x is the 2,4-DTBP concentration.

The percentage data recorded from persistence test of 2,4-DTBP in soil were checked for homogeneity of variance before subjected to two-way ANOVA where factor one is soil series whereas factor two is incubation period. The mean treatments were compared using Tukey test at 5% of significant level.

Conclusions

The findings in this study have revealed that 2,4-DTBP possesses potential pre-emergence herbicidal activity as a strong root inhibitor on tested bioassay species. 2,4-DTBP inhibited the root growth of *E. indica* in sandy loam soil by 100% at an application rate of 6.14 kg ai/ha. However, this potent root inhibitor was almost 100% degraded in silt loam soil (Renggam series) after 10 weeks, but more persistent in sandy loam soil (Rhu Tapai series soil). Consequently, it is suggested that the soil microbes have a great role in affecting the persistence of 2,4-DTBP where *E. indica* that treated with 2,4-DTBP under sterilized soil resulted in higher weed inhibition as compared those of under non-sterilized soil, implying that the presence of microbes in non-sterilized could cause a great loss of 2,4-DTBP phytotoxicity in soil. 2,4-DTBP can further be investigated for its formulation with various types of adjuvants in search of a safe herbicidal composition before it can be developed as a soil-applied natural herbicide for weed control.

Acknowledgement

This research was funded by Fundamental Research Grant Scheme (FRGS) Vote No. 59308, Ministry of Higher Education (MOHE), Malaysia.

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