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Fuqiang Zhao  
*Shenyang University*

Ping Wang  
*Stephen F Austin State University, Arthur Temple College of Forestry and Agriculture, wangp@sfasu.edu*

Rima D. Lucardi  
*USDA Forest Service*

Zushang Su  
*Stephen F. Austin State University, Arthur Temple College of Forestry and Agriculture, suz@sfasu.edu*

Shiyou Li  
*Stephen F. Austin State University, Arthur Temple College of Forestry and Agriculture, lis@sfasu.edu*

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Review

Natural Sources and Bioactivities of 2,4-Di-Tert-Butylphenol and Its Analogs

Fuqiang Zhao 1,2, Ping Wang 3, Rima D. Lucardi 4, Zushang Su 3 and Shiyou Li 3,*

1 College of Life Science and Bioengineering, Shenyang University, Shenyang 110044, Liaoning, China; zhaofuqiang@iae.ac.cn
2 CAS Key Laboratory of Forest Ecology and Management, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, Liaoning, China
3 National Center for Pharmaceutical Crops, Arthur Temple College of Forestry and Agriculture, Stephen F. Austin State University, Nacogdoches, TX 75962, USA; protectforest@hotmail.com (P.W.); fuq_zhao@126.com (Z.S.)
4 Southern Research Station, USDA Forest Service, 320 Green Street, Athens, GA 30602, USA; rima.lucardi@usda.gov

* Correspondence: lis@sfasu.edu

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Abstract: 2,4-Di-tert-butylphenol or 2,4-bis(1,1-dimethylethyl)-phenol (2,4-DTBP) is a common toxic secondary metabolite produced by various groups of organisms. The biosources and bioactivities of 2,4-DTBP have been well investigated, but the phenol has not been systematically reviewed. This article provides a comprehensive review of 2,4-DTBP and its analogs with emphasis on natural sources and bioactivities. 2,4-DTBP has been found in at least 169 species of bacteria (16 species, 10 families), fungi (11 species, eight families), diatom (one species, one family), liverwort (one species, one family), pteridiphyta (two species, two families), gymnosperms (four species, one family), dicots (107 species, 58 families), monocots (22 species, eight families), and animals (five species, five families). 2,4-DTBP is often a major component of violate or essential oils and it exhibits potent toxicity against almost all testing organisms, including the producers; however, it is not clear why organisms produce autotoxic 2,4-DTBP and its analogs. The accumulating evidence indicates that the endocidal regulation seems to be the primary function of the phenols in the producing organisms.

Keywords: 2,4-di-tert-butylphenol; 2,4-bis(1,1-dimethylethyl)-phenol (2,4-DTBP); 2,4-DTBP; analogs; natural source; bioactivities; autotoxicity; bacteria; fungi; plants; animals

Key Contribution: The comprehensive review of the biosources and bioactivities of 2,4-di-tert-butylphenol or 2,4-bis(1,1-dimethylethyl)-phenol (2,4-DTBP) and its analogs leads us to speculate that endocidal regulation is the primary function of these toxic phenols in the producing organisms.

1. Introduction

2,4-Di-tert-butylphenol or 2,4-bis(1,1-dimethylethyl)-phenol (2,4-DTBP) is a common natural product that exhibits potent toxicity against almost all testing organisms, including the producing species. The phenol has been well investigated in terms of its natural sources and bioactivities, but it has not been systematically reviewed. A basic question has never been addressed: why does an organism produces autotoxic 2,4-DTBP? This review has summarized the available references in both English and Chinese to date. It will provide some basic information to better understand the physiological and evolutionary roles of 2,4-DTBP in the producing organisms.
2. Natural Sources

2,4-DTBP is a lipophilic phenol reported in at least 169 species of organisms (see Table 1). 2,4-DTBP was found in 16 species of bacteria in 10 families, such as nitrogen-fixing cyanobacteria [1]; Gram-positive bacteria in hot spring, soils, and food [2–7] and Gram-negative bacteria in soil and freshwater [8–13]. Some bacteria are causal agents of infectious diseases in humans, e.g., Microcystis aeruginosa Kützing, a species of freshwater cyanobacteria that produce neurotoxins and peptide hepatotoxins [12]; and Vibrio alginolyticus Miyamoto et al., a marine bacterium causing otitis and wound infection [13]. The phenol has been identified from 11 fungal species of eight families, e.g., (Ditmar) Fr., and Didymium iridis (Eupatorium catarium (Griseb.) R.M.King & H. Rob. and (DC.) Danser [81]; and leaves of Carr., but not in the fallen leaves or decomposed leaves of the pine [32]. The phenol Pinus tabulaeformis The analysis also reported that 2,4-DTBP is a major component in the water extracts of fresh needles of phylum Porifera [71], centipede prevalent psychrophilic species (via distillation and methanol extracts of the cones and bark of Pinus yunnanensis Jacp. [88], and root exudate of sorghum [65]. It is also found in fungal 2,6-DTBP was detected in seeds of Jastropa curcas L. [76], rhizosphere soil of Boehmeria nivea (L.) Gauchid. [77], and algal Grateloupiptilia C. Ag. [78]. 2,6-DTBP was detected in seeds of Jastropa curcas L. [79] and Metaplexis japonica (Thum.) Makino [60]; flowers of Camellia sasanqua Thunb. [80], Aquilaria sinensis (Lour.) Gilg [45], and Taxillus chinensis (DC.) Danser [81]; and leaves of Chimonanthus spp. [82]. 3,5-DTBP was reported in flowers of Aesculus chinensis [83], fungal Coriolus versicolor [84], Aquilaria sinensis (Lour.) Gilg [45], whole plants of Hedychium lancea Thunb. [85], and seeds of Plukenetia volubilis L. [86]. 4-methyl-2,6-diterbutylphenol (butylated hydroxytoluene or dibutylhydroxytoluene, BHT) was found in the whole plants of Praxelis clematidea (Griseb.) R.M.King & H. Rob. and Eupatorium catarium Veldkamp [87], whole plants of Geum alleppicum Jacp. [82], and root exudate of sorghum [65]. It is also found in fungal Nectria [89]. The lipophilic phenol occurs in some plants, green algae, and cyanobacteria [90,91]. For example, the phenol was reported in rice [69] and Hedychium lancea Thunb. [85]. It was also found in the larval frass of sawyer beetles (Monochamus alternatus Hope) [92,93], and female frass of Chinese white pine beetles (Dendroctonus armandi Tsai et Li) [94]. It was believed to be produced by the host plant and is concentrated by larvae as a semiochemical compound [93]. However, a later experiment indicated that the phenol was present
in the beetle larvae only and not detected in the xylem samples of healthy trees, trees infected with blue-stain fungi, or the wall pupal chambers of *P. massoniana* [95]. 4-sec-butyl-2,6-diterbutylphenol was found in the stem of *Vernonia amygdalina* Del. [96]. 2,2′-methylenebis(6-tert-butyl-4-methylphenol) was found in the root exudate of sorghum [65]. It is noteworthy that phenols were detected in the sorghum root exudates in the second year of replantation but not in the following years [65].

![Structures of 2,4-DTBP and its natural analogs.](image1)

### Figure 1. Structures of 2,4-DTBP and its natural analogs.

#### 3. Antioxidant Activities

Some investigations on the antioxidant activities of this class of lipophilic phenols were focused on 2,4-DTBP (Figure 2, Table 2). Several in vitro methods for assaying the antioxidant activities have been used, for example, low density lipoprotein (LDL)-oxidation tools, including a thiobarbituric acid reactive substances (TBARS) assay, conjugated diene formation, the relative electrophoretic mobility (REM) of ox-LDL, apoB-100 fragmentation, radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, and copper chelating activity, such as in the copper-mediated TBARS assay (IC50: 8.20 mM), 2,2-azobis amidinopropane (AAPH)-mediated oxidation (IC50: 9.9 mM), and 3-morpholino-sydnonimine (SIN-1)-mediated oxidation (29% at 5.0 mM) [72]. 2,4-DTBP from sweet potato extract protects against hydrogen peroxide-induced oxidative stress in the pheochromocytoma cell line (PC12) and in mice [97]. Administration of 2,4-DTBP increased the alternation behavior in mice injected with amyloid-beta peptide (Ab1-42) [97].

![Bioactivities and potential applications of 2,4-DTBP and its natural analogs.](image2)

### Figure 2. Bioactivities and potential applications of 2,4-DTBP and its natural analogs.
The antioxidant activity of BHT was about twice as great as that of 2,4-DTBP because two ter-butyl groups in BHT protect the aromatic hydroxyl group, which forms a phenoxyl radical and donating a hydrogen atom that could quench active free radicals and stop the propagation of lipid peroxidation [98]. The additional ter-butyl group in BHT may also decrease the toxicity. As a result, BHT is one of most commonly used antioxidants for preserving food and feed, and is also listed as an antioxidant food additive by The U.S. Food and Drug Administration (FDA) and the European Union (EU) [99,100]. As an active ingredient from royal jelly, BHT can eliminate 75.86% of ultra-oxygen free radicals at 600 mg/L and 84.47% of the hydroxyl free radicals at 500 mg/L [101]. BHT decreased the Malondiadehyde (MDA) content and increased the superoxide dismutase (SOD) and glutathioneperoxidase (GSH-Px) content in rat liver and serum [101]. The antioxidant activity of BHT can be enhanced in combination use with synthetic 2-ter-butyl-4-methoxyphenol (BHA) and 2,4,6-tri-ter-butylphenl (TBP) [102]. BHT and BHA are fairly heat-stable, [1] but they have been found to exert a dual pro-oxidant and antioxidant action under certain conditions [102]. BHA can stimulate the peroxidase-dependent oxidation of BHT to form the potentially toxic BHT-quinone methide. Among several BHT metabolites, BHT-quinone methide (BHT-QM), 2,6-di-tert-butyl-4-hydroperoxyl-4-methyl-2,5-cyclohexadienone (BHT-OOH), and 3,5-di-tert-butyl-4-hydroxybenzaldehyde (BHT-CHO) have been reported to induce peroxides [102].

4. Anti-Inflammatory Activities

Lipopolysaccharide (LPS), the endotoxin found in the cell walls of Gram-negative bacteria, triggers inflammation by activating mononuclear phagocytes (monocytes and macrophages) and results in the production of various pro-inflammatory cytokines. LPS administration was observed to increase the expression of tumor necrosis factor alpha (TNF-α) interleukin IL-6 and IL-1b genes significantly, while 2,4-DTBP treatments were found to decrease the expression of all three genes in the RAW264.7 mouse macrophage cell line [103]. BHT has shown a slight anti-inflammatory activity on the expression of cyclooxygenase-2 (Cox2) and TNF-α genes upon stimulation with Porphyromonas gingivalis (Pg) fimbriae [102]. The combination of BHT and BHA at a molar ratio of 0.5–2 provides potent anti-inflammatory activity, as tested by gene-expression systems for Cox2 and TNF-α in RAW264.7 cells [102]. The anti-inflammatory activity may be attributable to complex synergistic antioxidant activity [102].

5. Cytotoxicities

2,4-DTBP showed a remarkable cytotoxicity against HeLa cells with an IC50 value of 10 µg/mL [6]. 2,4-DTBP exhibited superior effect in the induction of apoptotic genes in cancer cell lines, as did the standard drug Cisplatin [103]. 2,4-DTBP was found to significantly increase the expression of P53 and caspase 7 in both MCF-7 and A431 cell lines, and exhibited significantly higher activation of the P53 gene in MCF-7. Effect of 2,4-DTBP on caspase 7 gene expression was significantly greater in A431, while the effect appeared to be less pronounced in MCF-7 [103].

Based on hepatic and renal toxicity (histopathological changes and an increase in organ weight with blood biochemical changes) in rats, the respective no-observed-adverse-effect levels (NOAELs) for 2,4-DTBP were concluded to be 5 and 20 mg/kg/day [104]. Histologically, there were no obvious changes in uteri and vagina ovarietomized (OVX) CD1 mice between the 2,4-DTBP treatment and the control, and the uterotrophic effect of 2,4-DTBP was not observed in the range of 10 to 250 mg/kg using an oral gavage [105].

It has been reported that long-term and high quantities usage of BHT can induce liver tumors [106]. Due to their pro-oxidant activity, BHT-quinone and BHT-OOH have been reported to result in internucleosomal DNA fragmentation, which is the characteristic of apoptosis [107]. BHT-OOH was found through oxidative DNA damage directly, whereas BHT-quinone was found via DNA damage through H2O2 generation [107]. After an injection treatment, BHT can considerably increase the number of mitoses in epithelial cell populations from various parts of small intestinal crypts of mice [108]. The effect may be explained by the influence of BHT on the reserve pool of cells and the longevity of
individual stages of the mitotic cycle [108]. The BHA/BHT combination (molar ratio 1:1) has inhibited the expression of manganese superoxide dismutase (MnSOD) mRNA in HL60 cells and reversed the transcriptase-polymerase chain reaction (PCR)-activating caspases 3, 8, and 9 [109]. It may contribute to the synergistically antioxidant activity of the BHA/BHT combination and radical-induced formation of intermediates, such as quinone methide [109].

6. Insecticidal and Nematicidal Activities

2,4-DTBP exhibited significantly adulticidal, larvicidal, ovicidal, repellent, and oviposition-deterrent activities against the spider mite *Tetranychus cinnabarinus* [73]. The mites exhibited the highest run-off rate on bean leaf surfaces sprayed with 2,4-DTBP when applied at sublethal doses and moved toward surfaces that had not been sprayed with the compound, according to Pearson’s χ² test. The compound also showed nematicidal activity against Caenorhabditis elegans during fumigation or soil treatment at temperatures higher than 25 °C [110].

BHT showed larvicidal and ovicidal properties against warehouse beetles (*Trogoderma variabile* Ballion) and black carpet beetles (*Attagenus megatoma* (F.)) [111]. The compound also exhibited lethal insecticidal activity against other beetle species, such as saw-toothed grain beetles (*Oryzaephilus surinamensis* (L.)) and red flour beetles (*Tribolium castaneum* (Herbst)) [112]. The phenol may be used as a preservative in non-toxic aqueous pesticide [113]. It can be used as an adjuvant in a dienol formulation to stabilize p-mentha-1,3-dien-8-ol, an unstable monoterpenic alcohol, as a male-produced aggregation-sex pheromone to attract cerambycid beetles (*Paranoplium gracile* (Leconte)) of both sexes in field assays [114]. BHT has been as a component to repel female sawyer beetles [115].

7. Antibacterial Activities

Extracellular polymeric substances (EPS) play crucial roles in biofilm formation and biocorrosion, resulting in heavy economic loss in an industrial setup. 2,4-DTBP can modulate the secreted EPS of *Serratia marcescens*, which in turn could facilitate the disruption of biofilms, as well as favoring the diffusion of antimicrobials into the cell aggregates, resulting in the eradication of persistent biofilms [116]. 2,4-DTBP can be used to enhance the efficacy of conventional antibiotics. Intercellular communication in bacteria (quorum sensing (QS)) is an important phenomenon in disease dissemination and pathogenesis that controls biofilm formation. 2,4-DTBP controls QS-mediated biofilm formation and simultaneously increases the hydration of the cell wall, which results in reduced biofilm formation [13].

2,4-DTBP isolated from thermophilic *Bacillus licheniformis* in an Algerian hot spring showed bioactivity against two multidrug resistance bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus* in pure and mixed cultures that were investigated using a radial diffusion assay at 55 °C [2]. The phenol from *Bacillus*, in association with seaweed, was reported to exhibit a dose-dependent antibiofilm activity against group A *Streptococcus* bacterium [3].

8. Antiviral Activity

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) and plaque reduction assays showed that 2,4-DTBP exhibited significant anti-coxsackievirus B-3 (CVB-3) and anti-herpes virus type 2 (HSV-2) activities [117].

9. Antifungal Activities

2,4-DTBP was found to be effective against an agriculturally important root-rot fungus *Fusarium oxysporum* by inhibiting spore germination and hyphal growth [10]. During the fungal spore germination, 2,4-DTBP completely inhibited the germination by preventing the emergence of a normal germ tube and led to the abnormal branching and swelling of hyphae. In such a case, 2,4-DTBP may be binding with β-tubulin in microtubules, inhibiting their proliferation and suppressing their dynamic instability as the microtubules are the cytoskeletal polymers in eukaryotic cells and the loss of microtubules should negatively affect the growth rate of spore germination, with an expected reduction
in fungal growth in vitro. [10] 2,4-DTBP distinctly reduced the mycelial growth of Phytophthora capsici by approximately 50% at 100 µg/mL relative to the control [8]. The germinated seeds of pepper treated with 2,4-DTBP significantly reduced radicle infection by P. capsici without radicle growth inhibition [8].

2,4-DTBP had a significant inhibition effect on the mycelium growth at the early stage of culturing tomato leaf mold (Cladosporium fulvum) and 0.1 mmol/L of 2,4-DTBP had the best inhibition effect when the mycelium had grown for seven days [118].

The mycelium growth of Verticillium dahliae was drastically decreased with increasing concentrations of 2,4-DTBP (0.50 to 2.00 mmol/L.) [119].

2,4-DTBP can be produced in some species of Aspergillus [18], Penicillium [20,21], and Fusarium [23], but experiments showed the phenol could inhibit the growth of these fungi. Disc diffusion assays showed that 2,4-DTBP (2 mg/25 mL) prevented the fungal mycelial growth of Aspergillus niger, F. oxysporum, and Penicillium chrysogenum on wheat grains [6]. 2,4-DTBP produced from environmental bacterium Shewanella algae strain YM8 significantly reduced the mycelial growth and conidial germination in mold Aspergillus [11]. 2,4-DTBP could inhibit Aspergillus flavus mycelial growth 7 dpi on potatodextrose agar (PDA) medium at a 5 µg/L concentration and complete inhibition of mycelial growth was observed at 100 µg/L. At 200 µg/L, the compound completely inhibited the germination of conidia. The antimicrobial activity of 2,4-DTBP appeared to correlate with its antioxidative activity because it was able to inhibit the reactive oxygen species (ROS) production in both Aspergillus and Phytophthora cinnamomoni [120]. Thus, the phenol has potential in the development of biopreservatives and dietary antioxidants for food applications.

2,4-DTBP exhibited fungicidal potential at higher concentrations where fluconazole failed to act completely. Various antibiofilm assays and morphological observations revealed that 2,4-DTBP inhibited and disrupted biofilms of Candida albicans via the possible inhibition of hyphal development [101]. It also inhibited the production of hemolysins and phospholipases, and secreted aspartyl proteinase, which are the crucial virulence factors required for the invasion of C. albicans [121].

10. Phytotoxicity: Allelopathy and Autotoxicity

2,4-DTBP shows potential as a natural and environmentally friendly herbicide for weed management [122]. 2,4-DTBP from Chrysanthemum indicum inhibited seed germination and seedling growth of lettuce (Lactuca sativa var. ramosa Hort.), romaine lettuce (L. sativa L.), and rapeseed (Brassica napus L.) [65].

2,4-DTBP extracted from the rhizome of cogongrass (Imperata cylindrica (L.) P. Beauv.) was found to have allelopathic effects on the germination and seedling growth of weedy plants under soilless conditions; for instance, 2,4-DTBP at 0.1 mg/mL showed a 78–95% inhibition of root and shoot growth of beggar ticks (Bidens pilosa L.), leucaena (Leucaena leucocephala L. de Wit), and barnyardgrass (Echinochloa crus-galli (L.) Beauv.) [123]. Lab assays showed that leachates of cogongrass are toxic to ryegrass and lettuce, but not toxic to cogongrass [124]. However, another report showed that boiling water extracts of cogongrass rhizomes that contain catechol, chlorogenic acid, isochlorogenic acid, neochlorogenic acid, p-coumaric acid, p-hydroxybenzaldehyde, scopolin, and scopoletin not only significantly inhibited the seedling growth of five other plant species, but also suppressed cogongrass growth [125]. A later investigation indicated that 2,4-DTBP inhibited 100% of the seed germination and growth of cogongrass at the concentration of 0.1 mg/mL [123].

The phenol also showed toxicity on the root and leaf tissues of the grassy weed Leptochloa chinensis (L.) Nees and broadleaf weed Hedychium verticillata (L.) Lam [126] The phytotoxic effect of 2,4-DTBP on these two weeds became apparent at seven days and 14 days after treatment with symptoms of lamina wilting and necrosis, respectively [126]. After a 2,4-DTBP treatment, both had abnormal and much shorter root hairs compared to those of untreated plants. 2,4-DTBP reduced the shoot biomass growth of L. chinensis and H. verticillata by 50% when applied at concentrations of 50 and 200 µg/mL, respectively [122]. Chuah et al. found that 2,4-DTBP isolated from Napier grass (Pennisetum purpureum) exhibited potent herbicidal activity, whereby it completely prevented the root growth of L. chinensis in
soil at an application rate as low as 0.60 kg a.i. ha\(^{-1}\) [127]. 2,4-DTBP induces oxidative stress through the enhanced generation of reactive oxygen species, which cause lipid peroxidation, membrane damage, and the activation of antioxidant enzyme systems, and thus cause a great reduction in chlorophyll content, thereby decreasing chlorophyll fluorescence, transpiration, and the net photosynthetic rate in the leaf tissues [121]. 2,4-DTBP has potent herbicidal properties that can alter the chloroplast ultrastructure, thereby reducing physiological activity of these weedy plants [128]. The present findings imply that 2,4-DTBP may potentially be developed as a soil-applied natural herbicide for the control of \(L. \text{chinensis}\) and perhaps other weeds in an aerobic rice system [127,129].

It was reported that 2,4-DTBP from \(P. \text{massoniana}\) significantly inhibited the seed germination, seed viability, hypocotyl and radicle growth, and seedling growth of Masson’s pine at 0.25–1.0 mg/mL [33]. Another autotoxic study found that 2,4-DTBP had a toxic effect on microorganisms in the rhizosphere soil of hop (\(H. \text{lupulus}\) L.) and affected the photosynthesis and growth of hop seedlings [130,131]. 2,4-DTBP had a significant inhibitory effect on the plant immune system and seed germination of \(A. \text{macrolepida}\) [132]. 2,4-DTBP from root exudates of chilli pepper showed a medium inhibition against the seed germination and seedling growth of chilli pepper at more than 2 mmol/L [133]. The growth of eggplants was stunted at high concentrations (0.10–1.00 mmol L\(^{-1}\)) [104]. 2.5-DTBP is one of the compounds responsible for soil sickness in the field of \(B. \text{nivea}\) [77]. The results of a pot experiment indicated that 2,4-DTBP first significantly decreased and then increased the abundance of culturable bacteria, fungi, and actinomycetes of the rhizosphere soil after treatment [90,91]. 2,4-DTBP from the bulb of \(L. \text{davidii}\) var. \(w. \text{willmottiae}\) and \(F. \) display a synergetic effect on the \(F. \text{wilt}\) in the lily [134].

11. Conclusions

2,4-DTBP is a toxic lipophilic phenol reported in at least 169 species of organisms, such as bacteria (16 species of 10 families), fungi (11 species of eight families), diatom (one species), liverwort (one species), pteridophyta (two species of two families), gymnosperms (four species of one family), dicots (107 species of 58 families), monocots (22 species of eight families), and animals (five species of five families). To date, several analogs of 2,4-DTBP have been identified in bacteria, algae, fungi, plants, and insects, such as 2,5-DTBP, 2,6-DTBP, 3,5-DTBP, BHT, 4-sec-butyl-2,6-ditertbutylphenol, and 2,2’-methylenebis(6-tert-butyl-4-methylphenol).

The antioxidant and anti-inflammatory activities of 2,4-DTBP have been emphasized in many publications. More importantly, however, the phenol exhibited a broad toxicity in all testing organisms, including the producers; for example, cytotoxicity in human cells and animals, insecticidal and nematicidal activities, antimicrobial activities, and phytotoxicities. However, the available data could not explain why an organism produces such toxic 2,4-DTBP. The endocide theory hypothesizes that an organism is more sensitive to its own endogenous metabolites than external molecules and thus an endocidal compound commonly occurring in different species has a broad spectrum of toxicity or low selective activity [135]. 2,4-DTBP provides a good example. This phenol commonly occurs in diversified organisms and has a potent toxicity against almost all testing organisms.

The following aspects of 2,4-DTBP need to be addressed in future investigations. For example, 2,4-DTBP is usually a major component of volatile oils in many organisms, but its biosynthesis site is not known. A recent report showed that healthy rice plants had level of 2,4-DTBP similar to the plants of the same species following insect herbivory and viral infection [69], however, a carefully designed experiment is needed to determine whether the production of this phenol can be induced under stresses. Also, the presence of 2,4-DTBP analogs in organisms are often independent of 2,4-DTBP; it is important to elucidate the physiological role of these analogs in the producers. In addition, the bioactivities and potential applications of most analogs of 2,4-DTBP have not been well investigated, although BHT has been commonly used as antioxidants for preserving food and feed.
Table 1. Natural sources of 2,4-di-tert-butylphenol (2,4-DTBP).

| Family            | Biosource                              | Tissues                     | Ref.       |
|-------------------|----------------------------------------|-----------------------------|-----------|
| **Bacteria**      |                                        |                             |           |
| Bacillaceae       | *Bacillus licheniformis*               |                             | [2]       |
|                   | *B. subtilis* Ehrenberg                |                             | [3]       |
| Flavobacteriaceae | *Flavobacterium johnsoniae* (Stanier)  |                             | [8,9]     |
|                   | Bernardet et al.                       |                             |           |
| Microcystaceae    | *Microcystis aeruginosa* Kützing        |                             | [12]      |
|                   | *Arthrobacter* sp.                     |                             | [4]       |
| Nostocaceae       | *Nostoc* spp.                          |                             | [136]     |
|                   | *Anabaena oryzae* F.E. Fritsch         |                             |           |
|                   | *A. azotica* Ley                       |                             |           |
| Paenibacillaceae  | *Paenibacillus polymyxa* (Prazmowski)  |                             | [137]     |
|                   | Ash et al.                             |                             |           |
| Pseudomonadaceae  | *Pseudomonas monteilii* Elomari et al. |                             | [10]      |
| Shevanellaceae    | *Shevanella algae* Simidu et al.       |                             | [11]      |
| Streptococcaceae  | *Lactococcus* sp.                      | Cell-free supernatant       | [6]       |
| Streptomycetaceae | *Streptomyces globosus* Waksman         |                             | [4]       |
|                   | *S. mutabilis* Pridham et al.          |                             | [7]       |
| Vibrionaceae      | *Vibrio alginolyticus* Miyamoto et al.  | Cell-free culture supernatant | [13]     |
| **Fungi**         |                                        |                             |           |
| Agaricaceae       | *Agaricus bisporus* (J.E. Lange) Imbach|                             | [14]      |
| Bionectriaceae    | *Glomastix murorum* (Corda) S. Hughes  |                             | [17]      |
| Glomerellaceae    | *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. |           | [22]     |
| Nectriaceae       | *Fusarium tricinctum* (Corda) Saccardo |                             | [23]      |
| Omphalotaceae     | *Lentinus edodes* (Berk.) Pegler       | Caps and stipes             | [15]      |
| Polyporaceae      | *Trametes suaveolens* (L.) Fr.         |                             | [16]      |
| Tremellaceae      | *Cryptococcus albicus* (Saito) Skinner | Cell-free extract           | [24]      |
| Trichocomaceae    | *Aspergillus terreus* (Thom)           |                             | [18]      |
|                   | *Didymium iridis* (Ditmar) Fr.         |                             | [138]     |
|                   | *Penicillium flavigenum* Frisvad & Samson |                          | [20]      |
|                   | *Penicillium* sp.                      | Culture                     | [21]      |
| **Diatom**        |                                        |                             |           |
| Phaeodactylaceae  | *Phaeodactylum tricornutum* Bohlin     | Cells                       | [25]      |
| **Liverwort**     |                                        |                             |           |
| Marchantiaceae    | *Marchantia polymorpha* L.             | Whole thallus               | [26]      |
| **Pteridophyta**  |                                        |                             |           |
| Osmundaceae       | *Osmunda regalis* L.                   |                             | [27]      |
| **Pteridaceae**   | *Adiantum venustum* D. Don             |                             | [28]      |
| Gyumnasperms      |                                        |                             |           |
Table 1. Cont.

| Family          | Biosource                                | Tissues                          | Ref.     |
|-----------------|------------------------------------------|----------------------------------|----------|
| Pinaceae        | *Pinus kesya* var. *langbianensis* (A.chev.) Gavssen. | Cones                             | [31]     |
|                 | *P. massoniana* Lamb.                     | Rhizosphere soil                  | [33]     |
|                 | *P. tabulaeformis* Carr.                  | Needles                           | [139]    |
|                 | *P. yunnanensis* Franch.                  | Cones and bark                    | [129,140]|
| Dicots          |                                          |                                   |          |
| Amaryllidaceae  | *Allium fistulosum* L.                    | Root exudates                     | [141]    |
| Apiaceae        | *Anethum graveolens* L.                   |                                  | [142]    |
| Araliaceae      | *Centella asiatica* (L.) Urban            | Leaves                           | [143]    |
| Asclepiadaceae  | *Metaplexis japonica* (Thunb.) Makino     | Seeds                             | [60]     |
|                 | *Achroptilon repens* (L.) D.C.            | Aerial part                       | [145]    |
|                 | *Artemisia annua* L.                      |                                  |          |
|                 | *A. apiacea* Hance                        |                                  |          |
|                 | *A. japonica* Thunb.                      | Leaves                           | [34]     |
|                 | *A. capillaris* Thunb.                    |                                  |          |
|                 | *A. argyi* H.Lév. & Vaniot               |                                  |          |
|                 | *A. eriopoda* Bunge                       |                                  |          |
| Asteraceae      | *A. tschernieviana* Besser               | Aerial parts                      | [146]    |
|                 | *Atractylodes corona* (Nakai) Kitam       | Rhizomes                          | [147]    |
|                 | *A. macrocephala* Koidz                   | Rhizomes                          | [132]    |
|                 | *Chrysanthemum indicum* L.                | Leaves, stem, rot exudates, and rhizosphere soils | [63]     |
|                 | *Gynura ciusimhua* (D. Don) S. Moore      | Aerial parts                      | [148]    |
| Begoniaceae     | *Begonia malabarica* Lam.                 | Fruits and aerial parts           | [150]    |
| Boraginaceae    | *Heliotropium indicum* L.                 | Aerial parts                      | [151]    |
| Brassicaceae    | *Brassica oleracea var. capitata* F. Rubra | Leaves                           | [152]    |
|                 | *B. napus* L.                            | Seeds                             | [153]    |
| Cactaceae       | *Pereskia bleo* (Kunth) de Candolle       | Leaves                           | [154]    |
| Caeselpiniaceae | *Bauhinia variegata* (L.) Benth.          | Leaves                           | [155]    |
|                 | *Chimonanthus* Lindl.                     |                                  | [156]    |
|                 | *C. praeco* (L.) Link.                    |                                  |          |
|                 | *C. zhejiangensis* M.C. Liu               |                                  |          |
|                 | *C. salicifolius* S.Y. Hu                 |                                  |          |
|                 | *C. nitens* Oliv.                         |                                  |          |
|                 | *C. grammatus* M.C. Liu                   |                                  |          |
| Calycanthaceae  | *C. campanulatus* R.H.                    | Leaves                           | [82]     |
Table 1. Cont.

| Family       | Biosource                          | Tissues              | Ref.   |
|--------------|------------------------------------|----------------------|--------|
| Cannabaceae  | *Humulus lupulus* L.               | Rhizosphere soils    | [131]  |
| Capparaceae  | *Crataegus* religiosa G. Forst.    | Stems                | [157]  |
| Caprifoliaceae| *Lonicera maackii* (Rupr.) Maxim. | Fruits               | [64]   |
| Caricaceae   | *Carica papaya* L.                 | Seeds                | [158]  |
| Caryophyllaceae| *Spergularia marina* (L.) Besser | Aerial part          | [159]  |
| Combretaceae | *Terminalia travancorensis* Wight & Arn. | Bark                | [160]  |
| Convolvulaceae| *Ipomoea batatas* (L.) Lam. | Tubers               | [97]   |
| Cornaceae    | *Cornus officinalis* Sieb. Et Zucc. | Fruits              | [161]  |
| Cucurbitaceae| *Cucurbita moschata* (Duch. ex Lam.) Duch. ex Poiret | Fruits | [56]   |
| Crassulaceae | *Rhodiola imbricata* Edgew.       | Roots                | [162]  |
| Equisetaceae | *Equisetum arvense* L.            | Whole plant          | [163]  |
| Ericaceae    | *Rhododendron dauricum* L.        | Leaves               | [48]   |
| Euphorbiaceae| *Croton bonplandianum* Baill.     | Leaves               | [164]  |
|              | *Phyllanthus debilis* Klein ex Willd. | Leaves            | [165]  |
|              | *Sauropus rostratus* Miq.         | Leaves               | [55]   |
| Fabaceae     | *Albizia julibrissin* Durazz.     | Leaves and stems     | [49]   |
|              | *Dalbergia odorifera* T. Chen     | Wood                 | [166]  |
|              | *Humboldtia unijuga* Bedd.        | Roots                | [103]  |
|              | *Glycine max* (L.) Merr           | Root secretion       | [167]  |
|              | *Mucuna pruriens* (L.) DC.       | Seeds                | [168]  |
|              | *Vigna radiata* (L.) R. Wilczek   | Seeds                | [169]  |
| Gentianaceae | *Gentiana apiata* N. E. Br.       | Whole plants         | [46]   |
|              | *G. tibetica* King ex J.D. Hooker| Flowers              | [170]  |
| Hydrocharitaceae| *Hydrilla verticillata* (L.f.) Royle | Exudates            | [171]  |
| Juglandaceae | *Juglans regia* L.                | Root exudates        | [172]  |
| Lamiaceae    | *Sphenodesme inulocrata var. paniculata* (C. B. Clarke) Munir | Leaves            | [173]  |
|              | *Perilla frutescens* (L.) Britton | Leaves              | [174]  |
|              | *Salvia miltiorrhiza* Bunge       | Leaves and roots     | [175]  |
| Lauraceae    | *Cinnamomum longepaniculatum* (Gamble) N. Chao ex H. W. Li | Leaves      | [176]  |
|              | *C. loureirii* Nees               | Bark                 | [177]  |
|              | *Lindera aggregata* (Sims) Kosterm | Roots               | [178]  |
|              | *L. angustifolia* (W. C. Cheng) Nakai. *L. rubromeria* (Gamble) Rehder. | Xylem        | [179]  |
|              | *Persea americana* Mill.          | Roots                | [120]  |
| Loranthaceae | *Loranthus micranthus* L.         | Fresh leaves         | [180]  |
|              | *L. pentapetalus* Roxb.           | Leaves               | [181]  |
|              | *Viscum ovalifolium* Wallich ex Candolle | Leaves           | [181]  |
| Malvaceae    | *Cola nitida* (Vent.) Schott & Endl. | Fruits            | [182]  |
Table 1. Cont.

| Family              | Biosource                                      | Tissues                      | Ref. |
|---------------------|------------------------------------------------|------------------------------|------|
| Melastomataceae     | *Memecylon umbellatum* Burm. f                 | Leaves                       | [183]|  
| Menispermaceae      | *Tinospora cordifolia* (Willd.) Hook. f. &    | Embryogenic callus           | [184]|  
|                     | Thoms.                                         |                              |      |
| Myrtaceae           | *Eucalyptus globulus* L.                      | Leaves                       | [185]|  
|                     | *E. grandis* W. Hill ex Maiden                 | Root                         | [186]|  
|                     | *Eugenia dysenterica* D.C.                    | Fruits                       | [187]|  
| Nelumbonaceae       | *Nelumbo nucifera* Gaertn.                    | Rhizomes                     | [188]|  
| Oleaceae            | *Olea europaea* L.                            | Stems                        | [117]|  
| Paoniaeaceae        | *Paeonia lactiflora* Pall.                    | Root                         | [189]|  
| Papaveraceae        | *Eomecon chionanthera* Hance                  |                              | [67]|  
| Phyllanthaceae      | *Phyllanthus emblica* L.                      | Fruits                       | [61]|  
|                     | *Saurous rostratus* Miq.                      | Leaves                       | [55]|  
| Piperaceae          | *Piper nigrum* L.                             | Seeds                        | [190]|  
| Plumbaginaceae      | *Plumbago zeylanica* L.                       | Roots                        | [191]|  
| Polygonaceae        | *Calligonum polygonoides* L.                  | Fruits and stems             | [192]|  
|                     | *Polygonum viscosum* Buch-ham                 | Leaves                       | [193]|  
| Primulaceae         | *Lysimachia foenum-graecum* Hance             |                              | [194]|  
| Ranunculaceae       | *Aconitum carnichalae* Dibx.                 | Root                         | [68]|  
|                     | *Clematis comnata* D.C.                       | Whole plant                  | [195]|  
|                     | *Consolida regalis* Gray                      | Stem and leaves              | [196]|  
| Rosaceae            | *Chaenomeles sinensis* C.K. Schneid.          | Fruits                       | [197]|  
|                     | *Prunus persica* (L.) Batsch                  | Roots                        | [198]|  
|                     | *Rosa iberica* Stev.                          | Hips                         | [199]|  
|                     | *Sibiraea angustata* (Rehd.) Hand.-Mazz.      | Infructescence               | [54]|  
| Rubiaceae           | *Rubia cordifolia* L.                         | Stems                        | [200]|  
| Rutaceae            | *Zanthoxylum planispinum* Sieb. et Zucc.      | Litters                      | [201]|  
|                     | *Nauclea diderrichii* (De Wild. & T. Durand)  | Leaves                       | [202]|  
| Sapindaceae         | *Koelreuteria paniculata* Laxm.               | Leaves                       | [203]|  
| Saururaceae         | *Houttuynia cordata* Thunb.                   | Aerial part                  | [66]|  
| Scrophulariaceae    | *Verbascum phlomoides* L.                     | Flowers                      | [204]|  
| Solanaceae          | *Capsicum annum* L.                           | Root exudates                | [133,205]|  
|                     | *Solanum lycopersicum* var. cerasiforme       | Fruits                       | [206]|  
|                     | (Dunal) A.Gray                                |                              |      |
|                     | *S. melongena* L.                             | Root exudates                | [207]|  
|                     | *Withania coagulans* (Stocks) Dunal           | Leaves and micropropagated plant | [208]|  
| Styraeceae          | *Sinojackia sarcocarpa* L.Q. Lou              | Drupes                       | [209]|  
| Theaceae            | *Camellia sinensis* (L.) Kuntze               | Leaves                       | [210]|
### Table 1. Cont.

| Family       | Biosource                                      | Tissues                | Ref.  |
|--------------|------------------------------------------------|------------------------|-------|
| **Thymelaeaceae** | *Aquilaria sinensis* (Loureiro) Sprengel | Resin                  | [211] |
| **Urticaceae**   | *Boehmeria nivea* (L.) Gaudich.              | Rhizosphere soil       | [77]  |
|                | *Urtica dioica* L.                           | Leaves                 | [212] |
| **Violaceae**    | *Viola betonicifolia* Sm.                    | Whole plant            | [213] |
| **Vitaceae**     | *Ampelopsis grossedentata* (Hand.-Mazz.) W.T. Wang |                         | [214] |
| **Monocots**     |                                                |                        |       |
| **Araceae**      | *Amorphophallus campanulatus* (Dennst.) Nicolson | Tuber                  | [215] |
| **Arecales**     | *Cocos nucifera* L. (coconut)                | Fruit juice            | [216] |
| **Commelinaceae**| *Murdannia nudiflora* (L.) Brenan             | Whole plant            | [62]  |
| **Cyperaceae**   | *Cyperus rotundus* L.                        | Rhizomes               | [217] |
|                | *Helocharis dulcis* (Burm. f.) Trin.         | Rhizomes               | [136] |
|                | *Kyllinga triceps* Rottboll                  |                        | [218] |
| **Liliaceae**    | *Lilium davidii* var. *willmottiae* (E.H. Wilson) Raffill | Bulb                   | [134] |
| **Musaceae**     | *Musa* spp.                                   | Root                   | [219] |
| **Orchidaceae**  | *Dendrobium moniliforme* (L.) Sw.             | Flowers                | [220] |
|                | *Gastrodia elata* Blume                       | Rhizomes               | [125] |
| **Palmae**       | *Phoenix canariensis* Chabaud                 | Leaves                 | [221] |
|                | *Washingtonia filifera* (Lind.) H. Wendl. O’Brien |                        |       |
| **Poaceae**      | *Echinochloa crusgalli* (L.) Beauv            | Root exudates          | [222] |
|                | *Imperata cylindrica* (L.) Beauv              | Rhizome and root exudates | [123] |
|                | *Oryza sativa* L.                            | Root exudate           | [223] |
|                | *Pennisetum orientale* Rich.                 | Aerial part            | [47]  |
|                | *Pennisetum purpureum* Schumach.             | Culm and leaves        | [127,129] |
|                | *Phyllostachys pubescens* (Pradelle) Mazel ex J. Houz. | Fresh parenchyma   | [224] |
|                | *Sorghum bicolor* (L.) Moench                | Root exudate           | [65]  |
|                | *Spartina cynosuroides* (L.) Roth             | Fresh grass            | [225] |
|                | *Triticum durum* L.                          | Seeds                  | [226] |
| **Zingiberaceae**| *Zingiber cassumunar* Roxb.                  | Rhizomes and leaves    | [227] |
| **Animals**      |                                                |                        |       |
| **Mantidae**     | *Mantis ootheca*                              | Egg cases              | [75]  |
| **Mygalidae**    | *Zygomycale* sp.                              |                        | [71]  |
| **Scolopendridae**| *Scolopendra subspinipes* Leach               | Dried bodies           | [72]  |
| **Styelidae**    | *Styela clava* Herdman                        |                        | [74]  |
| **Tetranychidae**| *Tetranychus cinnabarinus* (Boisduval)        |                        | [73]  |
Table 2. The bioactivities of 2,4-di-tert-butyphenol (2,4-DTBP) and its analogs.

| Bioactivities                      | Chemical Name | Experimental Model | Treatment Doses | Cellular and Molecular Targets | Ref. |
|-----------------------------------|---------------|--------------------|-----------------|--------------------------------|------|
| Antioxidant Activities            | 2,4-DTBP      | TBARS assay        | IC_{50}: 8.20 mM| LDL-oxidation                  | [72] |
|                                   |               | Human plasma LDL   | IC_{50}: 9.9 mM | AAPH-mediated oxidation        | [72] |
|                                   |               | Human plasma LDL   | 5.0 mM          | SIN-1-mediated oxidation       | [72] |
|                                   |               | PheochromocytomPC12 cells and mice | 2–10 mg/100mL | Hydrogen-peroxide-induced oxidative stress | [97] |
|                                   |               | Mice injected with amyloid-beta peptide (Aβ1-42) | 5–40 mg/kg | Alternation behavior | [97] |
|                                   | BHT           | Ultra-oxygen-free radical | 600 mg/L | Radical scavenging | [101] |
|                                   |               | Hydroxyl-free radical | 500 mg/L | Radical scavenging | [101] |
|                                   |               | Liver and serum of rat | 100-800 mg/L | MDA, SOD, and GSH-PX content | [101] |
| Anti-Inflammatory Activities      | 2,4-DTBP      | RAW264.7 mouse macrophage cell line | 50 and 100 µg/mL | TNF-α, IL-6, and IL-1β genes | [103] |
|                                   | BHT           | RAW264.7 cells     | 10 µM           | Cox2 and TNF-α genes upon stimulation with Pg | [102] |
| Cytotoxicities                    | 2,4-DTBP      | HeLa cells         | IC_{50} value of 10 µg/mL | Cytotoxicity | [6] |
|                                   |               | MCF-7 and A431 cell lines | 50 and 100 µg/mL | P53 and caspase 7 generation | [103] |
|                                   |               | Rats               | 5 and 20 mg/kg/day | Respective no-observed-adverse-effect (NOAELs) | [104] |
|                                   |               | Uteri and vagina ovariectomized (OVX) CD1 mice | 10–250 mg/kg by oral treatment | Uterotrophic effect | [105] |
|                                   | BHT           | 32P-labeled DNA fragments | 50–500 µM | DNA damage | [107] |
|                                   |               | Small intestinal crypts of mice | Number of mitoses | | [108] |
|                                   |               | HL-60 and HSC-2 cells | 0.2–0.3 mM | Manganese superoxide dismutase (MnSOD) and reverse transcriptase-polymerase chain reaction (PCR) | [109] |
### Table 2. Cont.

| Bioactivities                      | Chemical Name | Experimental Model                      | Treatment Doses | Cellular and Molecular Targets                                                                 | Ref. |
|-----------------------------------|---------------|-----------------------------------------|-----------------|-------------------------------------------------------------------------------------------------|------|
| **Insecticidal and Nematicidal Activities** |               |                                        |                 |                                                                                                 |      |
|                                   | 2,4-DTBP      | Spider mite *Tetranychus cinnabarinus*  | LC$_{50}$ values of 1256.51, 625.39, and 743.64 ppm | Adulticidal, larvicidal, ovicidal, repellent, and oviposition-deterrent activities              | [73] |
|                                   |               | *Caenorhabditis elegans*                | 0.5–4 g/L       | Nematicidal activity                                                                           | [101]|
|                                   |               | *Trogoderma variabile*                  | 0.5 or 2.0%     | Larvical and ovicidal activity                                                                | [111]|
|                                   |               | *Oryzaephilus surinamensis* (L.), and *Tricholoma castaneum* (Herbst) | 10–45 mM       | Lethal insecticidal activity                                                                | [112]|
|                                   | BHT           | *Paranoplium gracile* (Leconte)         | 5% test solution | Stabilize a male-produced aggregation-sex pheromone                                            | [114]|
|                                   |               | Female *Monochamus alternatus*          |                 | Repellent activity                                                                            | [115]|
| **Antibacterial Activities**      | 2,4-DTBP      | Biofilm of *Serratia marcescens*        | 250–300 µg/mL   | Secreted extracellular polymeric substances, quorum sensing, and hydration of the cell wall | [13,116]|
|                                   |               | *Pseudomonas aeruginosa* and *Staphylococcus aureus* in pure and mixed culture |               | Antibacterial potency                                                                        | [2]  |
|                                   |               | Group A *Streptococcus* bacterium       | 16–48 µg/mL     | Antibiofilm activity                                                                           | [3]  |
| **Antiviral Activity**            | 2,4-DTBP      | Coxsackievirus B-3 (CVB-3) and herpes virus type 2 (HSV-2) | 6.32 ± 0.67 and 5.24 ± 0.82 | Antiviral activity                                                                            | [117]|
| **Antifungal Activities**         | 2,4-DTBP      | *Fusarium oxysporum*                    | 1–500 µg/mL     | β-tubulin in microtubules                                                                     | [10] |
|                                   |               | *Phytophthora capsici*                  | 100 µg/mL       | Mycelial growth                                                                                | [8]  |
|                                   |               | Pepper seed infected by *P. capsici*    | 1–100 g/mL      | Radicle infection                                                                              | [8]  |
|                                   |               | *Cladosporium fulvum*                   | 0.1 mmol/L      | Mycelium growth                                                                                | [118]|
|                                   |               | *Verticillium dahliae*                  | 0.50 to 2.00 mmol/L | Mycelium growth                                                                               | [119]|
|                                   |               | *Aspergillus niger, F. oxysporum* and *Penicillium chrysogenum* on wheat grains | 2 mg/25 mL | Fungal mycelial growth                                                                         | [6]  |
|                                   |               | *Aspergillus*                           | 5–200 µg/L      | Mycelial growth and conidial germination ROS production                                       | [11,120]|
|                                   |               | Biofilms of *Candida albicans*          | 2.5–100 µg/mL   | Hemolysins, phospholipases, and aspartyl proteinase                                             | [121]|

*Note:* LC$_{50}$ values are given as the concentration that inhibits 50% of the population.
Table 2. Cont.

| Bioactivities | Chemical Name | Experimental Model | Treatment Doses | Cellular and Molecular Targets | Ref. |
|---------------|---------------|---------------------|----------------|--------------------------------|------|
| Allelopathy   | 2,4-DTBP      | Seed and seedling of *Lactuca sativa* var. *ramosa* Hort. and *L. sativa* L. | 0–0.10 mmol/L | Seed germination and seedling growth | [63] |
|               |               | Seed and seedling of *Bidens pilosa* L. and *Leucaena leucocephala* L. de Wit | 0.1 mg/mL | Root and shoot growth | [123] |
|               |               | Root and leaf tissues of *Leptochloa chinensis* (L.) Nees and *Hedyotis verticillata* (L.) Lam | 50 and 200 µg/mL | Lamina wilting and necrosis, and root and shoot growth | [122, 126] |
|               |               | *L. chinensis* in soil | 0.60 kg a.i. ha\(^{-1}\) | Root growth | [127] |
|               |               | Leaf of weed plant | 2.5–100 µg/mL | Reactive oxygen species and chloroplasts | [121, 128] |
|               |               | Seed and seedling *Atractylodes macrocephala* | 0.1, 1, and 10 mmol/L | Plant immune system | [132] |
|               |               | Rhizosphere soil of *Litchi chinensis* Sonn. | Abundance | | [90] |
|               |               | Seed and seedling of *Imperata cylindrical* (L.) | 0.1 mg/mL | Seed germination and growth | [123] |
|               |               | Seed and seedling of *Masson’s pine* | 0.25–1.0 mg/mL | Seed germination, seed viability, hypocotyl and radicle growth, and seedling growth | [33] |
|               |               | Microorganism in the rhizosphere soil of *Hamulus lupulus* L. | 7.5 and 15 mmol/m\(^2\) | Photosynthesis and growth of hop seedlings | [130, 131] |
| Autotoxicity  | 2,4-DTBP      | Seed and seedling of *Brassica napus* L., *Echinochloa crus-galli* (L.) Beauv | 0.1 mg/mL | Root and shoot growth | [123] |
|               |               | Seed and seedling of *Brassica napus* L. | 0–0.10 mmol/L | Seed germination and seedling growth | [63] |
|               |               | Seed and seedling chilli pepper | More than 2 mmol/L | Seed germination and seedling growth | [133] |
|               |               | Seedling of eggplant | 0.10–1.00 mmol/L | Seedling growth | [104] |
|               |               | Bulb of *Fusarium* | *Fusarium* wilt in the lily | | [134] |
|               | 2,5-DTBP      | *Boehmeria nivea* | Soil sickness in the field | | [77] |

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