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Table: 3, Figs : 2

Allelopathic potential of *Sassafras tzumu* (Lauraceae) on seed germination and seedling growth of *Brassica rapa chinensis* L.

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ABSTRACT

The allelopathic potential of leaf extracts of *S. tzumu* on seed germination and seedling growth of *Brassica rapa chinensis* was determined and the extract constituents were determined using gas chromatography-mass spectrometer. The leaf extracts significantly decreased *B. rapa* seed germination and seedling growth at concentrations ≥ 0.8 g/ml. The seedling dry weight was unaffected. The root length of *B. rapa* seedlings was more sensitive to leaf extracts than stem elongation. Gas Chromatography-Mass Spectrometer (GC-MS) analysis detected 79 allelochemicals in the leaf extract of *S. tzumu*, which mainly consisted of phenol, alcohols and esters. The major components were 2,2'-Methylene-bis(6-tert-butyl-para-cresol) (CAS no: 119-47-1) and 1,4,5,8-Naphthaldiimide (CAS: 5690-24-4). The effects of these two chemicals on *B. rapa* seed germination and seedling growth were also examined. *Brassica rapa* was more sensitive to 1,4,5,8-Naphthaldiimide than to 2,2'-Methylene-bis(6-tert-butyl-para-cresol), suggesting that 1,4,5,8-Naphthaldiimide might be the major allelochemical in the ether fraction of the leaf extract of *S. tzumu*.

Keywords: Alcohols, allelochemicals, allelopathic potential, esters, germination, leaf extracts, phenols, *Sassafras tzumu*, seedling growth, weed.

INTRODUCTION

Sassafras tzumu (Lauraceae) is a perennial deciduous tree, widely distributed at an altitude of 150-1900 m, in the south of Changjiang River, China. It mostly grows in open and thick forests (5). It is ornamental tree with various leaf shapes. It grows fast, with a straight trunk of good timber quality and is suitable for the afforestation of broadleaved forests in South China. *S. tzumu* has broad applications in industrial products and medicines. Its seeds contain 20% oil, which is used for paint manufacture, while the bark and root contain 5%-8% of tannins, used for tanning leathers. Its root is traditional Chinese medicine to treat rheumatism, pain and hemiplegia (7,22). The diameter of the crown and the trunk of *S. tzumu* are relatively small. Thus, the growth of *S. tzumu* in pure forests is generally poor due to the extensive canopy closure of middle-aged forests. It generally grows in mixed forests with other species, because its pure forests are susceptible to *Dictyoploea japnica* (1).

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The interactions between *S. tzumu* and other species have been investigated. In general, *S. tzumu* is mixed in forests with *Phyllostachys pubescens*, *Pinus massoniana* or *Cunninghamia lanceolate* as it promoted the growth of these species (15,17). However, in mixed forests of *Phyllostachys pubescens*, a high density of *S. tzumu* (>735 trees/ha) inhibited the growth of bamboo (15). The authors explained this phenomenon as nutrients competition (15). However, *S. tzumu* could release allelochemicals, which inhibits the growth of *P. pubescens*. Moreover, the phytochemical analysis of the *Sassafras* genus indicated the presence of essential oils, neolignans and biphenyls (2,11,12,18). These chemicals may have allelopathic potential (3). In this context, our research was aimed to evaluate the allelopathic potential of leaf extracts of *S. tzumu* on seed germination and seedling growth of *B. rapa*. Furthermore, the chemical components of the ether fraction of the leaves of *S. tzumu* were identified using Gas Chromatography-Mass Spectrometer (GC-MS).

MATERIALS AND METHODS

I. Experimental materials

Leaves of *S. tzumu* were collected from November-December 2015 from 10 trees in the Jilingshan Park, Tunxi District, Huangshan City, Anhui Province, China. They were dried at 65^{o}C in an oven until their weight was constant. *B. rapa chinensis* seeds were purchased from Jiangxi Fengcheng Hangcheng Seed Company with germination rate ≥85%. The seeds were kept at 4 ^{o}C until used in bioassays.

II. Leaf extraction

Leaves of *S. tzumu* (20 g) were homogenized with a mortar in 10 ml methanol and a small amount of quartz sand until complete evaporation of the organic solvent. The powdered material was extracted and allowed to precipitate in 200 ml of 80% methanol for 24 h and the supernatant was collected. This step was repeated once. The supernatants were pooled, filtered through a 0.45-µm-pore-sized membrane and dried in oven at 35°C. The dry residue was suspended in 20 ml deionized water and further twice partitioned with 200 ml ether in a separating funnel. The ether fractions were pooled, dried at 35℃ and dissolved in 400 µl of dimethyl sulfoxide (DMSO). Next, the extract was diluted to 20 ml using deionized water to obtain a stock solution of *S. tzumu* leaves at 1 g per ml of deionized water. Finally, leaf extract was diluted to 0.2, 0.4, 0.6 and 0.8 g/ml of *S. tzumu* for assays. As control, 2% dimethyl sulfoxide in distilled water was used.

III. Bioassay of ether fraction

The ether fraction of leaf extract of *S. tzumu* was used to assay the germination and radicle growth of *B. rapa*. Seeds of *B. rapa* were soaked in distilled water for 6 h. Then, a layer of filter paper overlaying a layer of cotton wool was evenly positioned at the bottom of Petri dishes (Φ = 12 cm) (2,7,15). Next, 5 ml ether fraction of the leaf extract (0.2, 0.4, 0.6 and 0.8 g/ml) or distilled water (as control) was added to each Petri dish. For each treatment, 100 seeds of *B. rapa* were placed on the filter paper. Each treatment was repeated thrice. The Petri dishes were placed in incubator at 25°C**.** The germinated seeds were counted after 24 h, 48 h and 72 h. The root length, stem length, fresh weight and dry weight were measured after 72 h. Then, the germination rate, germination potential,

germination index and inhibitory index of root length, stem length, fresh weight and dry weight were calculated as follows:

Germination rate = Mean number of germinated seeds at 72 h/total number of seeds.

Germination potential = Mean number of germinated seeds at 48 h/total number of seeds.

$$\text{Germination index} = \sum(Gt/Dt),$$

Where, Gt: Mean germination number at different time points and Dt: Corresponding time (days) of germination.

Inhibitory index of root length, inhibitory index of stem length, inhibitory index of fresh weight, or inhibitory index of dry weight = [(average of root length, stem length, fresh weight, or dry weight in the control – average of root length, stem length, fresh weight, or dry weight in an experimental treatment) / average of root length, stem length, fresh weight, or dry weight in control] × 100%.

IV. Components in ether fraction of leaf extract

The chemical components of ether fraction of leaf extract were determined using GC-MS (Agilent GC7890A-MS5977B, CA, USA). A HP-5MS elastic quartz capillary column (0.25 μm × 30 μm × 0.25 mm) was used and helium was chosen as the carrier gas with a flow rate of 1 ml/min. The split ratio was 10:1. The initial temperature was 40℃, which was increased to 60℃ at a rate of 5℃/min and then maintained for 2 min. Subsequently, the temperature was further increased to 285℃ at the same rate and then maintained for 5 min. The injection volume was 0.5 μl using ether as the injection solvent. The temperature of the electron impact ion source was 230℃ and the electron energy was 70 eV. The mass scanning area was 35-450 amu. Identification was performed by comparison of the mass spectra with those stored in the NIST08 Mass Spectrum Library. The relative concentration of each constituent was calculated using the peak area normalization method (8, 20).

V. Allelopathic potential of major components in leaf extracts

The GC-MS analysis revealed that the major components in the ether extracts of *S. tzumu* withered leaves were 2,2'-Methylenebis(6-tert-butyl-4-methylphenol) (CAS: 5690-24-4) and 1,4,5,8-Naphthaldiimide (CAS: 119-47-1). To evaluate their allelopathic potential, these two chemicals were purchased from Shanghai Macklin Biochemical Co., Ltd (99% purity; Shanghai, China) and Tokyo Chemical Industry Co., Ltd., (99% purity; Japan), respectively, dissolved in DMSO and then diluted to 2% with distilled water. Three concentrations (0.1, 1 and 100 μg/mL) were prepared and 2% DMSO in distilled water was used as the control. The effects of 2,2'-Methylenebis(6-tert-butyl-4-methylphenol) and 1,4,5,8-Naphthaldiimide on germination and seedling growth of *B. rapa* were then bioassayed as previously described.

VI. Statistical analysis

The effects of leaf extract of *S. tzumu* on the germination (%), radicle growth, stem elongation, fresh and dry weight of *B. rapa* were analyzed by one-way ANOVA. Differences among means were analyzed by the least significant difference test. EC_{50} values were calculated using the probit method. All analyses were done using SPSS 21.0 program.

RESULTS AND DISCUSSION

Seed germination and seedling growth

The leaf extracts significantly affected the germination rate, germination potential, germination index, root length and stem length of *B. rapa*. In comparison to control, 0.6-0.8 g/ml leaf extracts significantly reduced the germination rate, germination potential and germination index ($P < 0.01$, Table 1). At concentration > 0.6 g/ml, the dry weight of seedlings was decreased. Root length and stem length were more sensitive to leaf extract. Even the low concentration (0.2 g/ml) extract, significantly inhibited the root length and stem length (inhibitory indices of 42.85% and 26.85%, respectively). At high concentration (0.8 g/ml), the inhibitory effects were more drastic (inhibitory index increased to 58.90% and 42.65% for root length and stem length, respectively (Figure 1). The EC_{50} values were 1.68 (95% confidence intervals: 1.29, 2.24), 1.49 (1.20, 2.20), 1.53 (1.28, 2.02), 0.54 (0.41, 0.71) and 0.72 (0.55, 1.14) g/ml for germination rate, germination potential, germination index, root length and stem length, respectively.

Table 1. Effects of leaf extracts of *S. tzumu* on the germination, seedling growth and biomass accumulation of *B. rapa chinensis*.

Parameter	Leaf extract concentration (g/ml)				
	0	0.2	0.4	0.6	0.8
Germination rate (%)	93.00	94.67	91.33	86.00	83.67
Germination potential (%)	84.33	85.67	83.33	75.33	73.67
Germination index (%)	73.16	74.39	72.11	66.33	64.72
Root length (mm)	19.83	11.33	10.22	8.98	8.15
Stem length (mm)	10.74	7.87	7.19	6.46	6.17
Dry weight (g)	0.172	0.169	0.170	0.153	0.159

Kong and Hu (14) and Wu (24) reported that allelopathic effects inhibited the plant growth by decreasing the germination rate, root length and quality of seedlings. *Sassafras albidum* released phytotoxins into the environment, which retarded the radical growth of several species (*Hacer negundo*, *Hacer saccharinum*, *Albizzia julibrissin*, *Pinus virginiana*, *Sorghum halepense*, *Triodia dentata* and *Ulmus americana*) (9). The bark oil of *S. albidum* inhibited the seed germination of *Lactuca sativa* (IC_{50} = 1834 μg/ml) and *Lolium perenne* (IC_{50} = 1848 μg/ml) and radicle elongation of *L. sativa* (6). In the present study, similar results were observed. The leaf extracts of *S. tzumu* significantly inhibited the germination rate, germination potential and germination index of *B. rapa* seeds as well as the stem length and root length of *B. rapa* seedlings, suggesting that the chemical components in *S. tzumu* might have allelopathic potential.

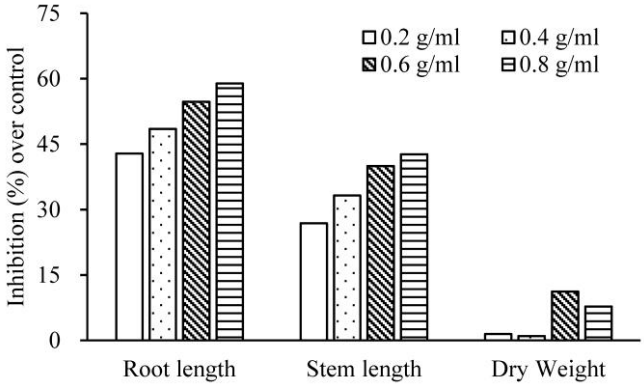

Figure 1. Inhibitory effects of leaf extracts of *S. tzumu* on the seedling growth of *B. rapa chinensis*.

Allelochemicals from different donor plants had various effects on the same plant (16). In the present study, root length was most sensitive to the exposure of leaf extracts of *S. tzumu*, followed by stem length, with an EC_{50} value was 0.54 (0.41, 0.71) and 0.72 (0.55, 0.14), respectively. These results were consistent with those of Zhang *et al*. (26) showing that the inhibitory effects of leaf extracts of *Carya illinoinensis* were more remarkable on the underground parts compared to aboveground parts (shoot) of the target plants. These phenomena might be explained by the long exposure time of the root to the test solution compared with the stem, as the radicle develops earlier than the epicotyl during germination. Another explanation might be that the radicle is more sensitive to allelochemicals than the epicotyl (27).

Organic chemical components of leaf extracts

The allelochemicals of plants are generally secondary metabolites consisting of various complex components. Both inhibitory and promoting chemicals might be present in crude extracts of plants (25). In addition, allelochemicals generally possess multi-ecological functions (13). The isolation and identification of allelochemicals are important to study the allelopathy of plants (23), but the trace amounts of allelochemicals often hinder their collection, isolation and identification. The development of modern techniques for chemical analysis and identification provides effective technical support for the isolation and identification of allelochemicals. For example, GC-MS is a commonly used method (4,19) and can be used to identify approximately 20% of organic compounds (21). In the present study, the leaf extracts of *S. tzumu* were analyzed using GC-MS. The results revealed 79 compounds in the leaf extracts, which mainly consisted of alcohols, esters, phenol, alkenes, aldehydes and ketones. The relatively abundant components were phenol, 2,2'-Methylenebis(6-tert-butyl-4-methylphenol) (CAS no: 119-47-1) and 1,4,5,8-Naphthaldiimide (CAS: 5690-24-4) (Table 2).

Table 2. Chemical components of withered leaves extracts of *S. tzumu*.

Peak No.	RT	AP	Chemical name	CAS number
1	28.546	0.0999	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	000489-39-4
2	30.572	1.0474	Butylated Hydroxytoluene	000128-37-0
3	31.737	0.0766	Silane, (1,1-dimethylethyl)dimethyl[(1-methylene-2-propenyl)oxy]-	080738-05-2
4	32.219	0.3408	2(1H)-Pyridinethione, 3-hydroxy-	023003-22-7
5	32.812	0.0612	Cyclopentanecarboxamide, N-(2-fluorophenyl)-	1000308-60-7
6	32.995	1.6776	Caryophyllene oxide	001139-30-6
7	33.09	0.0873	2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	1000144-10-2
8	33.345	0.0784	2-(4a,8-Dimethyl-2,3,4,4a,5,6-hexahydronaphthalen-2-yl)propan-1-ol	1000189-03-1
9	33.828	0.0716	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-	019888-34-7
10	34.504	0.0977	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-	017334-55-3
11	34.739	0.1237	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5.beta.-ol	019431-80-2
12	35.39	0.2651	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, cis-	056362-87-9
13	35.738	0.0435	Cycloisolongifolene, 8,9-dehydro-	1000151-28-0
14	35.963	0.1711	Caryophyllene oxide	001139-30-6
15	36.33	0.0764	Spiro[5.6]dodecane	000181-15-7
16	37.79	0.0615	Spiro[4.5]decan-6-one	013388-94-8
17	37.913	0.0325	6-Tridecene, 7-methyl-	024949-42-6
18	38.289	0.036	trans-Z-.alpha.-Bisabolene epoxide	1000131-71-1
19	38.609	0.0616	Diepicedrene-1-oxide	1000156-11-0
20	39.489	0.4727	Benzene, 1-(1,1-dimethylethyl)-4-methoxy-	005396-38-3
21	39.847	0.0848	Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)-	000513-23-5
22	40.48	1.3237	Cyclopentanecarboxaldehyde, 2-methyl-3-methylene-	1000154-24-0
23	40.634	0.2397	2-(1-Hydroxycycloheptyl)-furan	115754-89-7
24	40.956	0.2057	1-Methoxy-3-(2-hydroxyethyl)nonane	070928-44-8
25	41.109	0.1724	2-Pentadecanone, 6,10,14-trimethyl-	000502-69-2
26	41.548	0.0544	13-Tetradece-11-yn-1-ol	1000131-00-4
27	41.964	0.08	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	102608-53-7
28	42.125	0.4217	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	1000191-00-6
29	43.293	0.105	Anthralin	000480-22-8
30	43.613	0.111	Dibutyl phthalate	000084-74-2
31	43.988	0.19	Estra-1,3,5(10),9(11)-tetraen-17-one, 3-methoxy-	001670-49-1
32	44.87	0.0577	Germacyclopentane, 1-chloro-	004554-75-0
33	45.8	0.0595	9,12-Octadecadienoic acid, methyl ester	002462-85-3

34	46.306	0.1409	6-Octadecenoic acid, methyl ester, (Z)-	002777-58-4
35	46.376	0.0508	Pyrrolidin-2-one, 5-[3-heptanon-1-yl]-	116454-69-4
36	46.461	0.1268	Phytol	000150-86-7
37	46.982	0.1097	Octadecanoic acid, methyl ester	000112-61-8
38	47.539	0.1066	4,5-Diphenyl-2-imidazolethiol	002349-58-8
39	47.683	0.0861	4-Methyl-2-(3,7,11-trimethyl-dodeca-2,4,6,8,10-pentaenylide neamino)-pent-4-enenitrile	1000189-17-1
40	47.832	0.0378	3H-Indazol-3-one, 1,2-dihydro-2-(4-methoxyphenyl)-5-methyl-	028561-69-5
41	48.114	11.27	Benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetrone	005690-24-4
42	48.23	5.2356	Condyfolan-16-carboxylic acid, 2,14,16,19-tetradehydro-, methyl ester, (14E)-	004939-81-5
43	48.823	0.7005	Equilenin	000517-09-9
44	48.937	0.3533	m-Bis(p-methoxyphenoxy)benzene	013118-91-7
45	49.048	0.1529	(2-Methylene-cyclohexyl)-phenyl-methanol	130447-40-4
46	49.454	2.0931	Cyclopenta[d]anthracene-8,11-dione, 1,2,3,3a,4,5,6,6a,7,12-decahydro-3-isopropyl-6-methylene-	1000188-44-2
47	49.786	7.1992	4,8,12,16-Tetramethylheptadecan-4-olide	096168-15-9
48	49.91	0.9097	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, methyl ester, [1R-(1.alpha.,4a.beta.,10a.alpha.)]-	001235-74-1
49	50.149	1.31	9,10-Anthracenedione, 1,8-dihydroxy-3-methyl-	000481-74-3
50	50.253	1.365	4,8,12,16-Tetramethylheptadecan-4-olide	096168-15-9
51	50.795	0.0766	(7-Methyl-4,6,6a,7,8,10a-hexahydroindolo[4,3-fg]quinolin-9-yl)-methanol	1000192-61-1
52	51.085	0.8139	3-(3-Methoxyphenyl)propenenitrile, 2-(diethoxyphosphonyl)-	107846-75-3
53	51.622	51.3387	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	000119-47-1
54	52.078	1.2123	6-(2-Oxiranylmethyl)-1,2,3,4,5,11-hexahydro-9H-cyclohepta[b]quinolin-9-one	1000148-41-8
55	52.228	0.9537	1-Nonadecene	018435-45-5
56	52.428	0.1142	3-(3-Methoxyphenyl)propenenitrile, 2-(diethoxyphosphonyl)-	107846-75-3
57	52.479	0.0759	3-(3-Methoxyphenyl)propenenitrile, 2-(diethoxyphosphonyl)-	107846-75-3
58	52.565	0.37	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	004376-20-9
59	53.557	0.0052	3-(3-Methoxyphenyl)propenenitrile, 2-(diethoxyphosphonyl)-	107846-75-3
60	54.852	0.2103	Eicosane	000112-95-8
61	56.06	0.1081	3-(3-Methoxyphenyl)propenenitrile, 2-(diethoxyphosphonyl)-	107846-75-3
62	56.187	0.0603	3-(3-Methoxyphenyl)propenenitrile, 2-(diethoxyphosphonyl)-	107846-75-3
63	56.533	0.2149	Cannabinol	000521-35-7
64	56.776	0.2553	Cannabinol	000521-35-7
65	57.247	0.1425	1-Tricosene	018835-32-0
66	57.756	0.0524	2-(3,7-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-purin-1-ylmethyl)-benzonitrile	1000300-28-4
67	57.94	0.0769	3-(3-Methoxyphenyl)propenenitrile, 2-(diethoxyphosphonyl)-	107846-75-3
68	59.163	0.1226	Cannabinol	000521-35-7

69	59.222	0.0675	3-(3-Methoxyphenyl)propenenitrile, 2-(diethoxyphosphonyl)-	107846-75-3
70	59.468	0.2328	1H-Indole, 1-ethyl-5-methoxy-2-(4-methoxyphenyl)-3-methyl-	091444-33-6
71	59.801	0.0447	3-(3-Methoxyphenyl)propenenitrile, 2-(diethoxyphosphonyl)-	107846-75-3
72	60.163	0.5311	9-Tricosene, (Z)-	027519-02-4
73	60.493	0.373	Vitamin E	000059-02-9
74	60.98	0.1053	9-Tricosene, (Z)-	027519-02-4
75	62.286	0.175	Campesterol	000474-62-4
76	63.023	0.0503	Benzoic acide, 4-(4-nitrophtalimidyl)-, 4-tertbutylphenyl ester	1000223-69-6
77	63.85	1.7296	16-Hexadecanoyl hydrazide	002619-88-7
78	64.21	1.0274	Pregn-5-en-3-ol, 21-bromo-20-methyl-, (3.beta.)-	055103-80-5
79	64.377	0.1234	gamma-Sitosterol	000083-47-6

RT: retention time; AP: area percentage.

Effects of 2,2'-Methylenebis(6-tert-butyl-4-methylphenol) and 1,4,5,8 -Naphthaldiimide on *B. rapa*

Compared with the control, 0.1-100 μg/ml 1,4,5,8-Naphthaldiimide significantly decreased the germination rate, germination potential and germination index of *B. rapa* and 1-100 μg/ml 1,4,5,8-Naphthaldiimide significantly reduced stem length and root length. Similarly, 1-100 μg/ml 2,2'-Methylene-bis(6-tert-butyl-para-cresol) significantly inhibited the germination potential and germination index and as little as 100 μg/ml 2,2'-Methylene-bis(6-tert-butyl-para-cresol) significantly inhibited the stem length and root length (Table 3, Figure 2). The EC_{50} of 1,4,5,8-Naphthaldiimide on germination potential, germination index, root length and stem length was 58.71 (37.41, 97.34), 104.29 (73.23, 183.92), 88.40 (70.38, 117.28) and 185.84 (131.80, 346.04), respectively. The EC_{50} of 2,2'-Methylene-bis(6-tert-butyl-para-cresol) on germination potential, germination index and root length was 65.89 (57.16, 76.08), 101.83 (91.27, 115.24) and 209.62 (144.95, 412.87) μg/ml, respectively (Table 3).

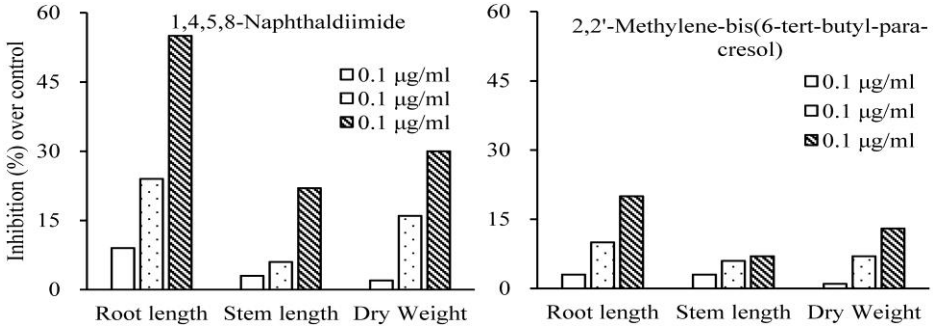

Figure 2. Inhibitory effects of chemical components [1,4,5,8-Naphthaldiimide (CAS: 5690-24-4) and 2,2'-Methylene-bis(6-tert- butyl-para-cresol) (CAS: 119-47-1)] on the seedling growth of *B. rapa chinensis*.

Taken together, both 1,4,5,8-Naphthaldiimide and 2,2'-Methylene-bis(6-tert-butyl-para-cresol) inhibited the germination and root growth of *B. rapa*. The root length was more sensitive to both chemicals than stem length. These results were consistent with the effects of the leaf extracts of *S. tzumu*, suggesting that both these chemicals might function as allelochemicals in these leaf extracts. Furthermore, 1,4,5,8-Naphthaldiimide displayed more effective allelopathic potential on *B. rapa* than 2,2'-Methylene-bis(6-tert-butyl-para-cresol) because the EC_{50} value of 1,4,5,8-Naphthaldiimide for RL was lower than that of 2,2'-Methylene-bis(6-tert-butyl-para-cresol).

Table 3. Effects of 1,4,5,8-Naphthaldiimide (CAS: 5690-24-4) and 2,2'-Methylene-bis(6-tert-butyl-para-cresol) (CAS: 119-47-1) on the germination and seedling growth of *B. rapa chinensis*.

Indices	0 μg/ml	0.1 μg/ml	1 μg/ml	100 μg/ml	EC_{50} (μg/ml)
1,4,5,8-Naphthaldiimide					
GR (%)	99.20	96.00 (-3.22)	88.20 (-11.1)	87.40 (-11.9)	Not available
GP (%)	98.00	85.40	53.00	27.80	58.71 (37.41, 97.34)
GI (%)	82.07	74.70	55.90	43.03	104.29 (73.23, 183.92)
RL (mm)	23.29	21.20	17.61	10.49	88.40 (70.38, 117.28)
SL (mm)	13.86	13.44	13.03	10.87	185.84 (131.80, 346.04)
DW (mg)	2.23	2.02	1.77	1.51	Not available
2,2'-Methylene-bis(6-tert-butyl-para-cresol)					
GR (%)	99.20	98.40	97.80	97.2	Not available
GP (%)	98.00	94.00	88.40	20.20	65.89 (57.16, 76.08)
GI (%)	82.07	79.80	76.80	42.50	101.83 (91.27, 115.24)
RL (mm)	23.29	22.41	20.93	18.46	209.62 (144.95, 412.87)
SL (mm)	13.86	13.59	13.07	12.83	Not available
DW (mg)	2.23	2.17	2.04	1.97	Not available

GR: germination rate, GP: germination potentiality, GI: germination index, RL: root length, SL: stem length, DW: dry weight.

The 2,4-dichlorophenoxyacetic acid (2,4-D), (+)-2-*cis*-4-*trans*-abscisic acid (*cis*-ABA), 1-*O*-*cis*-cinnamoyl-*β*-D- glucopyranose (CG), 6-*O*-(4-hydroxy-2-methylene-butyroyl)-1-*O*-*cis*-cinnamoyl-*β*-D-glucopyranose (BCG) and *cis*-cinnamic acid (*cis*-CA) were used as herbicides. Using the filter paper method, the EC_{50} of these chemicals on root elongation of *B. rapa* ranged from 10^{-4} to 10^{-2} mM (10). In the present study, the EC_{50} value of 1,4,5,8-Naphthaldiimide and 2,2'-Methylene-bis (6-tert-butyl-para-cresol) on the root length of *B. rapa* seeding was 88.40 (70.38, 117.28) μg/ml [equal to 0.33 (0.26-0.44)

mM] and 209.62 (144.95, 412.87) μg/ml [equal to 0.61 (0.43-0.12) mM], respectively. These EC_{50} values were much higher than the above-mentioned commercial herbicides. Thus, there is no potential for the development of these two chemicals as herbicides.

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