



## Composition of the essential oil of *Lepidium meyenii* (Walp.)

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### Abstract

The essential oil profile of maca (*Lepidium meyenii*) obtained from Lima, Peru, was examined. Steam distillates of the aerial parts of *L. meyenii* were continuously extracted with pentane and the pentane extracts analyzed by GC/MS. Retention indices and mass spectral data were used to identify 53 oil components. Phenyl acetonitrile (85.9%), benzaldehyde (3.1%), and 3-methoxyphenylacetonitrile (2.1%) were the major components of the steam distilled oil. The oil of *L. meyenii* was tested for phytotoxic, cyanobactericidal, and antitermite activity. The oil was selectively toxic towards the cyanobacterium *Oscillatoria perornata* compared to the green alga *Selenastrum capricornutum*, with complete growth inhibition at 100 µg/ml. Mortality of the Formosan subterranean termite, *Coptotermes formosanus*, was numerically, but not significantly, higher when held on filter paper treated with maca oil. At 1% (w/w), maca oil also appeared to act as a feeding deterrent to termites. Several minor components of the essential oil of maca including 3-methoxyphenylacetonitrile and benzylthiocyanate were significantly active against the Formosan termite. This is the first report on the essential oil composition of *L. meyenii*. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Lepidium meyenii*; Brassicaceae; Maca; Essential oil composition; Cyanobacteria; Algae; Plant; Termite; *Coptotermes formosanus*; Bioactivity

### 1. Introduction

*Lepidium meyenii* (Walpers) (maca) is a member of the Brassicaceae family, which includes many edible species including brussels sprouts, watercress, radish, and turnip. This family is one of 16 families known to contain glucosinolates, which give rise to compounds that have been investigated for a range of known bioactivities including phytotoxic, antifungal, antibacterial, and insecticidal effects (Fahey et al., 2001). *L. meyenii* itself is an edible root plant found in the Andean regions of Peru. It is sold as a nutritional supplement and is gaining popularity as a botanical extract for, among other things, its enhancement of energy and sexual performance. Little is known, however, about the secondary constituents of *L. meyenii*. The water, carbohydrate, lipid, protein, fiber, inorganic salt, amino acid, fatty

acid, mineral, and sterol content of the tuber has been studied (Dini et al., 1994; Comas et al., 1997). To our knowledge, the composition of the essential oil of *L. meyenii* has not been previously reported and is the focus of this paper. Since part of our mission is to discover plant constituents that can be used in pest management, the phytotoxic, cyanobacterial, and termatocidal activities of the oil and some of its major components were also studied.

### 2. Results and discussion

The identity, retention index, retention time, and the percent composition of the oil of *Lepidium meyenii* are presented in Table 1. Percent composition is presented as relative area (peak area relative to total peak area). Fifty-three compounds were identified in the oil of maca, accounting for over 94% of the composition of the oil. The oil is characterized by a very disagreeable odour. The oil constituents are consistent with those of

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a member of the Brassicaceae family. Among the identified compounds were 22 phenolic compounds (93.5%) including 2 nitrogen (85.9%), 14 oxygen (4.5%), 1 sulfur

Table 1  
Constituents of the oil of *Lepidium meyenii*

Compound	RT	KI	%RA
Mesityl oxide	2.62	792	0.1
Furfural	3.03	830	0.2
<i>E</i> -2-Hexenal	3.32	851	0.1
<i>Z</i> -3-Hexenol	3.37	855	t
<i>p</i> -Xylene	3.65	872	t
2,4-( <i>E,E</i> )-Hexadienal	4.33	908	t
2-Acetylfuran	4.37	910	t
Benzaldehyde	5.55	961	3.1
6-Methyl-5-hepten-2-one	6.27	986	t
2-Pentylfuran	6.45	992	t
Mesitylene	6.54	994	t
2,4-Heptadienal	6.97	1009	t
2-Acetylthiazole	7.19	1016	t
Limonene	7.66	1032	t
Benzyl alcohol	7.73	1034	t
Benzenecetaldehyde	8.04	1043	0.5
2-Acetylpyrrole	8.58	1059	t
Acetophenone	8.83	1065	t
Benzenemethanethiol	9.39	1080	0.4
<i>o</i> -Guaiaicol	9.707	1088	t
Methyl benzoate	9.939	1093	t
Linalool	10.18	1098	t
<i>cis</i> -Thujone	10.33	1102	t
Phenylethyl alcohol	10.62	1111	t
Phenylacetoneitrile	11.80	1142	85.9
Benzene acetic acid methyl ester	13.29	1177	t
<i>p</i> -Methylacetophenone	13.52	1182	t
<i>p</i> -Cymen-8-ol	13.62	1184	t
<i>m</i> -Methoxybenzaldehyde	14.10	1194	0.3
Myrtenal	14.22	1197	0.1
<i>trans</i> -Carveol	15.13	1219	t
Indole	18.21	1289	t
2-Methoxy-4-vinylphenol	19.17	1310	0.1
Benzylthiocyanate	20.35	1339	t
Benzylisothiocyanate	21.29	1361	0.6
3-Methoxyphenylacetoneitrile	21.81	1372	2.1
<i>E</i> - $\alpha$ -Ionone	24.17	1427	t
Geranyl acetone	25.24	1453	0.1
<i>E</i> - $\beta$ -Ionone	26.60	1484	0.1
<i>n</i> -Pentadecane	27.31	1500	t
<i>E,E</i> - $\alpha$ -Farnesene	27.62	1508	t
<i>n</i> -Heptadecane	35.21	1700	t
Methyl tetradecanoate	36.11	1725	t
Benzyl benzoate	37.40	1761	t
<i>n</i> -Nonadecane	42.38	1900	t
Methyl hexadecanoate	43.24	1926	0.1
Ethyl hexadecanoate	45.52	1994	t
Methyl linoleate	48.68	2092	t
Methyl linolenate	48.87	2098	0.2
Phytol	49.31	2112	0.1
<i>n</i> -Docosane	51.98	2200	t
<i>n</i> -Tricosane	54.92	2300	t
<i>n</i> -Tetracosane	57.74	2400	t

RT=retention time on a DB-5 column in minutes. KI=Kovats retention indices as determined on a DB-5 column using the homologous series of *n*-hydrocarbons. RA=relative area (peak area relative to total peak area). t=trace (<0.05%).

(0.4%), 1 nitrogen and oxygen (2.1%), and 2 nitrogen and sulfur (0.6%) substituted phenolic compounds. Additionally, 5 aliphatic esters (0.3%), 6 terpenoid compounds (0.2%), and trace amounts of 6 different straight chain hydrocarbons were found. None of the remaining unidentified compounds accounted for more than 0.5% of the total area. The main component, phenylacetoneitrile, is known to be a degradation product of benzyl glucosinolate (Saarivirta, 1973) in other plants including water cress (*Lepidium sativum*). Some of the other phenolics including benzyl thiocyanate and benzyl isothiocyanate have also been reported as degradation products of benzylglucosinolates (Saarivirta, 1973). Benzaldehyde, benzyl alcohol, and benzyl mercaptan have been reported as possible thermally-formed secondary products from the isothiocyanate and thiocyanate, respectively (Gil and MacLeod, 1980). Allyl isothiocyanate, also reported in *L. sativum* (Gil and MacLeod, 1980) and as a degradation product of benzylglucosinolates in *Alliaria petiolata* (Vaughn and Berhow, 1999), was not detected in the essential oil of *L. meyenii*. 3-Methoxybenzyl isothiocyanate has been shown previously to be the major breakdown product of glucolimnanthin, the main glucosinolate in *Limnanthes alba* (Vaughn et al., 1996). Whether or not these compounds are derived from glucosinolates in *L. meyenii* is still unknown, and though the chemistry of glucosinolates is not within the scope of this paper, their chemistry in the Brassicaceae, as well as the biosynthetic pathways involved in their formation, has been well studied (Fahey et al., 2001).

The oil of *L. meyenii* was tested for phytotoxic, anti-termite, and anticyanobacterial activity, and showed no phytotoxic activity under the conditions used. Glucosinolate 3-methoxyphenylacetoneitrile (2.1% RA in the oil) has been previously reported to be a phytotoxin (Vaughn et al., 1996) on tests that were carried out at higher concentrations (~40 mM) than the highest concentration tested in our assays (1 mg/ml of crude oil).

Termite (*Coptotermes formosanus*) mortality was numerically, but not significantly higher on maca oil treatments compared with untreated filter paper (data not shown). At 1%, maca oil appeared to act as a feeding deterrent to termites, with only 1 mg of filter paper consumed compared with 15.9 mg control consumption (data not shown). No activity was observed when phenylacetoneitrile, the major component of the oil, was tested (Table 2). Good activity was observed, however, with some of the minor components, namely benzylthiocyanate, 3-methoxyphenylacetoneitrile, and  $\beta$ -ionone (Table 2). Dose response results for these are shown in Table 3. The activity of benzylthiocyanate and 3-methoxyphenylacetoneitrile, the two most active, appears to be lost fairly quickly. Seven days after 100% mortality had been observed, which occurs within 24–48 h with benzylthiocyanate and 3-methoxyphenylacetoneitrile,

reloading of the test sample wells with fresh termites produced no significant differences when compared to controls. To the best of our knowledge, neither 3-methoxyphenylacetone nitrile nor benzylthiocyanate have previously been reported to have antitermite activity. Benzylthiocyanate and other thiocyanates have been reported previously as having activity against other insects, supposedly through enzymatic generation of hydrogen cyanide (Zsolnai, 1974; Ohkawa et al., 1972; Bakry et al., 1968). The toxic activity of *Limnanthes alba* seeds to fall armyworm and European corn borer has been ascribed to the glucosinolate 3-methoxybenzylisothiocyanate (Bartelt and Mikolajczak, 1989)

and the insecticidal activity of benzylthiocyanate and phenylacetone nitrile against *Musca domestica* and *Rhizopertha dominica* discussed (Peterson et al., 1998). Phenylacetone nitrile has been correlated with the metabolic generation of hydrogen cyanide by other organisms (Potter et al., 2001). Whether generation of hydrogen cyanide plays a role in the activity of 3-methoxyphenylacetone nitrile or benzylthiocyanate against termites is not presently known.

Screening of *L. meyenii* oil against the cyanobacterium (blue-green alga) *Oscillatoria perornata* and the green alga *Selenastrum capricornutum* indicated potential as a selective cyanobactericide. In west Mississippi,

Table 2  
Cumulative% mortality of *C. formosanus* on filter paper treated with individual components of maca essential oil

Treatment	% Mortality (mean±S.D.) <sup>a,b</sup>					
	Days					
4 µmol	1	4	7	9	15	21
Mesityl oxide	0D	1.7±2.9C	1.7±2.9B	3.3±5.8B	3.3±5.8B	8.3±10.4B
<i>p</i> -Xylene	0D	1.7±2.9C	1.7±2.9B	1.7±2.9B	1.7±2.9B	3.3±2.9B
Benzaldehyde	0D	0C	0B	1.7±2.9B	1.7±2.9B	1.7±2.9B
2,4-Heptadienal	1.7±2.9D	1.7±2.9	1.7±2.9B	3.3±2.9B	5.0±5.0B	15.0±13.2B
Phenylacetone nitrile	0D	0C	0B	0B	0B	0B
Myrtenal	0D	1.7±2.9C	1.7±2.9B	1.7±2.9B	1.7±2.9B	1.7±2.9B
2-Methoxy-4-vinylphenol	3.3±5.8D	3.3±5.8C	3.3±5.8B	3.3±5.8B	3.3±5.8B	3.3±5.8B
3-Methoxyphenylacetone nitrile	83.3±16.1B	100.0±0.0A	100.0±0.0A	100.0±0.0A	100.0±0.0A	100.0±0.0A
<i>b</i> -Ionone	43.3±28.9	66.7±45.4B	71.7±49.1A	71.7±49.1A	71.7±49.1A	71.7±49.1A
Benzylthiocyanate	100.0±0.0A	100.0±0.0A	100.0±0.0A	100.0±0.0A	100.0±0.0A	100.0±0.0A
Untreated	0D	0C	0B	1.3±2.5B	1.3±2.5B	1.3±2.5B

<sup>a</sup> Twenty workers (> 3rd instar) /1 soldier per rep., 3 reps., 3 colonies.

<sup>b</sup> Means within a column/treatment with the same letter are not significantly different, SNK:  $P < 0.05$ .

Table 3  
Cumulative% mortality of *C. formosanus* on filter paper treated with different amounts of individual components of maca essential oil

Treatment	% Mortality (mean±S.D.) <sup>a,b</sup>				
	Days				
	1	3	11	16	21
<i>Benzylthiocyanate</i>					
0.5%	0A	0A	1.7±2.9A	23.3±17.6A	33.3±33.3A
0.1%	0A	0A	1.7±2.9A	6.7±7.6AB	11.7±12.6A
0.05%	0A	0A	1.7±2.9A	1.7±2.9AB	1.7±2.9A
Untreated	0A	0A	0A	0B	0A
<i>β</i> -Ionone					
0.5%	0A	0A	0A	5.0±8.7A	5.0±8.7A
0.1%	0A	0A	0A	0A	0A
0.05%	0A	0A	1.7±2.9A	1.7±2.9A	1.7±2.9A
Untreated	0A	0A	0A	0A	0A
<i>3-Methoxyphenylacetone nitrile</i>					
0.5%	100.0±0.0A	100.0±0.0A	100.0±0.0A	100.0±0.0A	100.0±0.0A
0.1%	0B	0B	0B	0B	0B
0.05%	0B	0B	0B	0B	1.7±2.9B
Untreated	0B	0B	0B	0B	0B

<sup>a</sup> Twenty workers (> 3rd instar) /1 soldier per rep., 3 reps., 3 colonies.

<sup>b</sup> Means within a column/treatment with the same letter are not significantly different, SNK:  $P < 0.05$ .

the cyanobacterium *O. perornata* (Skuja), a producer of the musty-odor compound 2-methylisoborneol (MIB), is attributed with being the major cause of musty off-flavor in farm-raised catfish (van der Ploeg et al., 1995). Off-flavor in channel catfish (*Ictalurus punctatus*) raised in the southeastern United States creates an unpalatable and, therefore, unmarketable product that results in large economic losses to the industry. Green algae are not associated with such undesirable metabolites and are also preferable to cyanobacteria in catfish production ponds because they are better oxygenators of the water and a better base for aquatic food chains (Paerl and Tucker, 1995). Therefore, the discovery of safe compounds that selectively kill cyanobacteria would greatly benefit the channel catfish industry. Toxic activity (lowest-observed-effect concentration, LOEC) and complete inhibition (lowest-complete-inhibition concentration, LCIC) of *L. meyenii* oil towards *O. perornata* was observed at 100 µg/ml (Fig. 1A). For *S. capricornutum*, on the other hand, no activity was observed even at concentrations of 1000 µg/ml (Fig. 1B). Phenylacetonitrile, the main oil constituent of *L. meyenii* was also tested for cyanobactericidal activity and was found to be selectively toxic against the cyanobacterium *O. perornata* with an LCIC of 117 µg/ml (Fig. 2A and B). Phenylacetonitrile may be the component in *L. meyenii* oil causing toxicity towards *O. perornata* since 100 µg/ml of the oil (85.9% RA phenylacetonitrile) was completely inhibitory, but not 10 µg/ml, and 117 µg/ml phenylacetonitrile was completely inhibitory, but not 11.7 µg/ml. Phenylacetonitrile is not a promising compound for use as a selective algicide in catfish aquaculture ponds due to the high level required to kill *O. perornata* (i.e., cost) and potential toxicity to non-target organisms such as catfish.

### 3. Experimental

#### 3.1. Plant material

Air dried samples of the aerial parts of *L. meyenii* were obtained from Lima, Peru, through the American Mercantile Co, Memphis, TN. A voucher specimen (NP-1051) was placed in the University of Mississippi herbarium located in Oxford, MS.

#### 3.2. Essential oil isolation and chemical characterization

Steam distillation and analyses of the oil were conducted as previously described (Tellez et al., 2000; Adams, 1995) on 100.17 g of plant material. Analyses were performed by GC–MS (EI, 70 eV) with a DB-5 column (30 m×0.25 mm fused silica capillary column, film thickness 0.25 µm) using He as carrier gas (1 ml/min), 1 µl injection size and a programmed (injector

temp.: 220 °C, transfer line temp.: 240 °C, initial column temp.: 60 °C, final column temp.: 240 °C, 3 °C/min) temp. run (Tellez et al., 2000; Adams, 1995). Identification of oil components was performed by a comparison of mass spectra with literature data, and by a comparison of their relative retention times with those of authentic compounds, or by comparison of their retention indices with those in the literature (Adams, 1995, 2001). The relative amounts (RA) of individual components of the oil are expressed as percent peak area relative to total peak area. These percentages are used only for analysis of the oil components and are not used in the bioassays, where appropriate units for each assay are used. A clear yellow oil was obtained in a yield of 59.8 mg (0.06% of fr. wt).

#### 3.3. Algicidal assays

A rapid bioassay (Schrader et al., 1997) was used to determine the LOEC and the LCIC of *L. meyenii* oil towards isolates of the cyanobacterium (blue-green alga) *Oscillatoria perornata* and the green alga *Selenastrum capricornutum*. A 96-well quartz microplate (Hellma Cells, Inc., Forest Hills, New York) was used to perform the bioassay since the pentane loading solvent is incompatible with polystyrene microplates.

#### 3.4. Phytotoxicity assays

Bioassays for phytotoxic activity were carried out as previously reported for lettuce (*Lactuca sativa* cv. ‘Iceberg’) and bentgrass (*Agrostis stolonifera* cv. ‘pencross’) in 24-well plates (Dayan et al., 1999) except for the following: *n*-Pentane was used as the transfer solvent and the solvent allowed to evaporate on the filter paper prior to adding water on the seeds. An added control with *n*-pentane was used to account for possible effects of trace amounts of solvent.

#### 3.5. Termatocidal assays

Three colonies of *C. formosanus* were obtained from field sites in New Orleans, Louisiana. *C. formosanus* colonies were collected from the United States Department of Agriculture’s Southern Regional Research Center or from the University of New Orleans, New Orleans, LA. *C. formosanus* were collected from bucket traps (Su and Scheffrahn, 1986) and maintained on stacked, water soaked, spruce (*Picea* sp.) slats (10×4×0.5 cm) in plastic containers (13×13×4 cm) maintained under conditions of ca. 100% R.H. and 26.6 °C. Termites were identified using keys for soldier identification (Sheffrahn and Su, 1994; Su et al., 1997).

Maca oil was dissolved in acetone and 100 µl of this solution was pipetted onto 2.5 cm dia. Whatman No. 1

filter paper. The acetone was allowed to evaporate from the filter paper for several hours. Filter paper was treated at 1.0, 0.1, and 0.01% (w/w) maca oil. Treated filter paper disks were placed in plastic Petri dishes (35×10 mm) and moistened with 100  $\mu$ l water. Twenty *C. formosanus* undifferentiated workers (3rd instar or greater as determined by size) and a single soldier were placed on each treatment. The absence of a soldier(s) causes

the initiation of physiological process in which a certain number of workers become soldiers. This process imparts a metabolic cost to the worker changing its response to exogenous compounds. It also causes behavioral changes including a cessation of feeding preceding their molt to presoldier. The addition of a soldier(s) in the bioassay reduces this source of variability. Treatments were replicated 3 times and held separate from

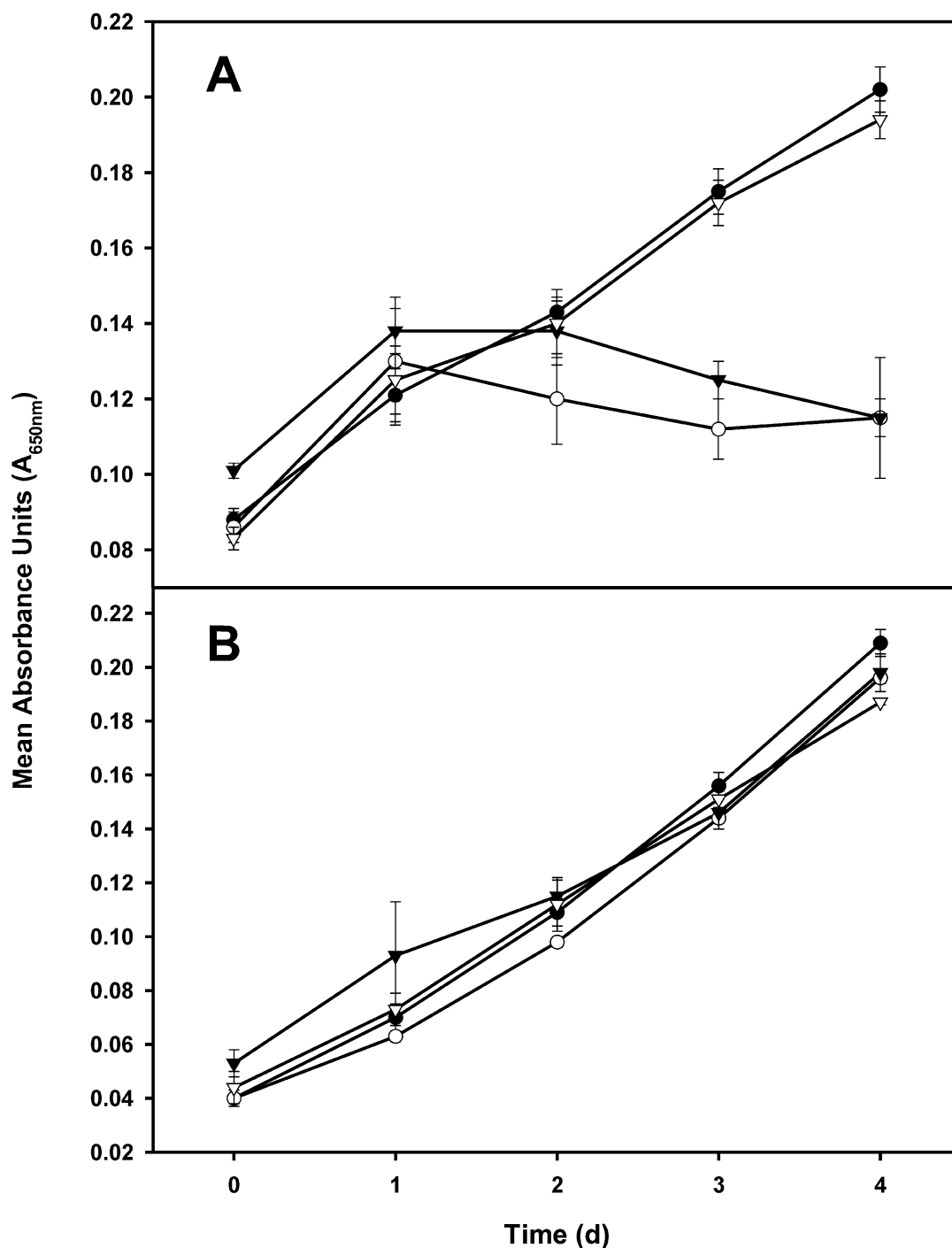


Fig. 1. Effect of different concentrations of maca oil on the growth of (A) *O. perornata* and (B) *S. capricornutum*: (●) control; ( $\nabla$ ) 10  $\mu$ g/ml; ( $\blacktriangledown$ ) 100  $\mu$ g/ml; and (○) 1000  $\mu$ g/ml maca oil.

other treatments. Each replicate originated from a different *C. formosanus* colony. Petri dishes were maintained at ca.100% RH and 27 °C. Filter paper disks receiving water alone served as controls. It was previously determined that the various solvents alone had no discernible effect on termite mortality or consumption.

Daily termite mortality was evaluated for 19 days. Cumulative daily mortality (mean and standard deviation)

was calculated for each treatment. Treatments were compared using ANOVA and means separated with Student–Newman–Keuls (SNK) multiple range test, following transformation to arcsine square root percent mortality (SAS Institute, 1990). Actual percent mortality is reported in the tables. Cellulose consumption was determined gravimetrically and reported as total consumption over the entire experiment with treatments

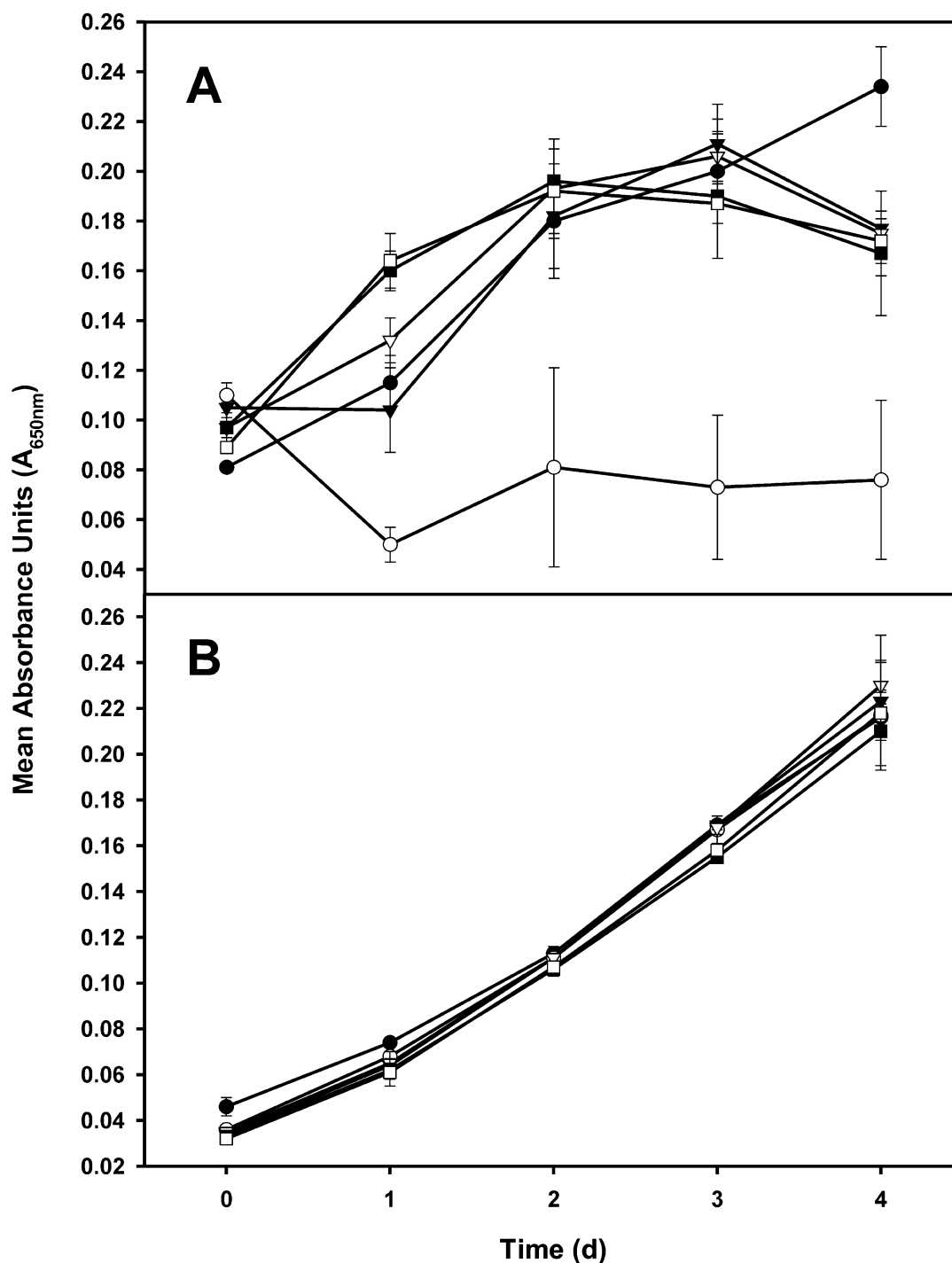


Fig. 2. Effect of different concentrations of phenylacetonitrile on the growth of (A) *O. perornata* and (B) *S. capricornutum*: (●) control; (□) 0.0117 µg/ml; (■) 0.117 µg/ml; (▽) 1.17 µg/ml; (▼) 11.7 µg/ml; and (○) 117 µg/ml phenylacetonitrile.

compared using ANOVA and means separated with SNK multiple range test (SAS Institute, 1990).

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