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Potential of garlic oil to control biological contamination of Chlamydomonas cultures by the ciliate Oxytricha Peer-reviewed author version

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36	Keywords separated by ' - '	<i>Chlamydomonas</i> Ciliate control - N	culture - Oxytricha contamination - Biological pesticides - licroalgal biomass protection	
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# Potential of garlic oil to control biological contamination of *Chlamydomonas* cultures by the ciliate Oxytricha

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#### 11 Abstract

Facilities for the production of microalgal biomass often suffer large losses in productivity as a result of biological contamination 12of cultures by ciliates, unicellular protozoans that feed on microalgae. Garlic oil is a low-cost natural product that is known to be 1314active against several protozoans. In this study, we investigated whether garlic oil can be used to control ciliate contamination in microalgal cultures, using the ciliate Oxytricha and the microalga Chlamydomonas as a model system. Low doses of garlic oil (5-15 $10 \text{ mg L}^{-1}$ ) were capable of eradicating the ciliate Oxytricha from a contaminated Chlamydomonas culture within 1 day without 16influencing the productivity of the *Chlamydomonas* culture. The LD<sub>50</sub> of garlic oil to the ciliate (3 mg L<sup>-1</sup>) was 19 times lower 17than the  $LD_{50}$  to the microalgae, which implies a low risk to the microalgal culture in case of overdosing. Analysis of the garlic oil 18 indicated that it was composed mainly of polysulfides, with the main compound being diallyl disulfide. Diallyl disulfide had a 19lower toxicity to the ciliate  $(LD_{50} \ 14 \ mg \ L^{-1})$  than garlic oil, indicating that dially disulfide is not the main active compound in 20garlic oil against the ciliate. Because garlic oil has a low cost, is already approved for use in agri- and aquacultures, has a low 21toxicity to humans, and is biodegradable, it may offer a sustainable solution to control biological contamination by ciliates in 2223microalgal cultures.

Keywords Chlamydomonas culture · Oxytricha contamination · Biological pesticides · Ciliate control · Microalgal biomass
 protection

# 2627 Introduction

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Microalgae are attracting worldwide attention as a novel feed-28stock for the production of biofuels as well as high-value 2930 products (Borowitzka 2013). Production of microalgae today is mostly done in open cultivation systems or raceway ponds, 31but because these are open to the atmosphere, these systems 32 33 are highly susceptible to biological contamination by pest species such as parasites, weed microalgae and grazers 34(Wang et al. 2013; Day et al. 2017). These pest species have 35

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high growth rates and can cause a complete crash of the cul-36ture in a matter of days (Peng et al. 2015). Hence, control of37biological contamination is one of the key challenges to de-38velop reliable large-scale production of microalgal biomass.39

An important class of biological contaminants is the cili-40 ates: protozoa that feed on microalgae. Several methods have 41 been proposed to control ciliate contamination in microalgal 42 cultures (for a recent overview, see Day et al. 2017). 43Historically, low doses of formaldehyde have been used to 44 control ciliate contamination (Rothbard 1975) but the use of 45formaldehyde as a biocide is prohibited in Europe (European 46 Commission 2011). Ciliates can be controlled by a combina-47tion of ammonium addition and a pH increase to raise the 48concentration of free ammonia, but this approach often results 49in a decline in the productivity of the microalgal culture be-50cause free ammonia is not only toxic to ciliates but also to 51microalgae (Karuppasamy et al. 2018). Inhibitors that are spe-52cific to protozoans such as quinine sulfate have been used with 53success but would be expensive to apply at scale (Moreno-54Garrido and Cañavate 2001). Pulsed electric field treatment 55can be used to kill ciliates but its use is restricted to freshwater 56microalgae (Kempkes 2017). 57 Q4

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In this study, we explore the use of garlic (Allium sativum) 58to control ciliate contamination in microalgal cultures. Garlic 59extracts and the organosulfur compounds they contain are 60 61well known to have an inhibitory activity against infectious 62 protozoa such Entamoeba histolytica, Giardia intestinalis, or Trypanosoma species and polysulfides isolated from garlic 63 64 have been used successfully to treat protozoan infections in 65 China (Lun et al. 1994; Ankri et al. 1997; Harris et al. 2000). Extracts from garlic are also commercially used in aquaculture 66 67 and ornamental fish to control protozoan diseases (Lee and 68 Gao 2012). Addition of garlic to the diet of ruminants is also 69 known to reduce numbers of intestinal ciliates (e.g., McAllister and Newbold 2008). Therefore, we hypothesize 70that garlic may have potential to control ciliate contamination 71in microalgal cultures. Extracts from garlic are relatively easy 72to prepare and may therefore offer a low-cost solution to this 73problem. An important advantage of garlic over other chem-7475ical control methods is that as a natural product, it is free from 76extensive legal regulation and complies with consumer pref-77 erences for natural over synthetic products.

We used the ciliate Oxytricha and the microalga 78Chlamydomonas as a model system to test the potential of 79 80 garlic for control of ciliate contamination a protozoan grazer of microalgae. Oxytricha is frequently reported as a biological 81 82 contaminant in outdoor microalgal mass cultures (Cho et al. 83 2017; Wang et al. 2017). We tested a garlic oil extract obtained from an international chemical supplier (Sigma). We also an-84 alyzed the chemical composition of the extract and tested the 85 activity of the main active compound present in this extract, 86 diallyl disulfide, to identify the main active compound in the 87 crude garlic extract towards the ciliate Oxytricha. Many treat-88 89 ments to control ciliate contamination also inhibit the growth of microalgae when applied at a higher dosage (e.g., Rothbard 90 1975; Karruppasamy et al. 2017; Kempkes 2017). Therefore, **Q5**91 we evaluate the toxicity of garlic oil to the ciliate Oxytricha as 92well as the microalga Chlamydomonas. 93

#### 94 Materials and methods

#### 95 Cultivation of microalgae and ciliates

The chlorophyte Chlamydomonas reinhardtii SAG 77.81 and 96 97 the hypotrich ciliate Oxytricha sp. that feed on Chlamydomonas were used as model systems to evaluate the 98 efficacy of a crude garlic extract and the active compound 99100 diallyl disulfide to control ciliate contamination in microalgal cultures. Chlamydomonas was maintained in 2 L batch cul-101 tures in Wright's cryptophyte (WC) medium (Guillard and 102Lorenzen 1972) in a temperature-controlled room  $(20 \pm$ 103 1042 °C) at a light intensity of 80  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup> and a light-dark cycle of 16:8 h. Exponential growth phase cultures 105were used in all experiments. Growth of Chlamydomonas 106

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cells was monitored spectrophotometrically at optical density107750 nm ( $OD_{750}$ ) (Griffiths et al. 2011). Optical density was108calibrated against gravimetric dry weight measurements109(using Whatman GF/F glass microfiber filters) and against cell110density (determined microscopically using a Bürker counting111chamber).112

The ciliate Oxvtricha was isolated from a eutrophic reser-113 voir close to the KU Leuven campus in Kortrijk, Belgium. 114Individual cells of the ciliate were picked up using a fine 115Pasteur pipette, washed several times in sterile WC medium, 116and then transferred into a Chlamydomonas culture. The 117 Oxvtricha cultures were maintained in 6-well microtiter plates 118 in 3 mL volume cultures. The cultures were renewed every 1193 days by transferring 0.5 mL of the ciliate suspension to a 120well containing 2 mL of fresh WC medium and 0.5 mL of a 121stationary phase Chlamydomonas culture (containing biomass 122concentration expressed as  $OD_{750}$  of 0.7 or 2 × 12310<sup>7</sup> Chlamydomonas cells mL<sup>-1</sup>). Exponentially growing 124Oxytricha cultures with an abundance of about 1254000 cells mL<sup>-1</sup> were used as an inoculum in all experiments. 126The ciliate was identified as Oxytricha based on morpholog-127ical criteria using Berger (1999) (Fig. 1). 128

# Evaluation of ciliate inhibition by garlic extract and diallyl disulfide

An essential oil obtained from Chinese garlic by steam distillation as well as the most abundant active chemical in this extract (diallyl disulfide) were obtained from an international chemical supplier (Sigma-Aldrich, Belgium). The garlic oil and diallyl disulfide were dissolved in methanol to enhance their solubility in the microalgal culture medium. This resulted in the addition of maximum 0.6% methanol to the culture 137



**Fig. 1** Photograph of the hypotrich ciliate *Oxytricha* sp. containing *Chlamydomonas* cells in its food vacuoles (red arrow)

medium. This should not affect the results as exploratory experiments had demonstrated that both ciliates and microalgae
can tolerate a methanol concentration of up to 2% (data not
shown).

142Exploratory tests were performed to determine the concentration range at which the garlic oil and diallyl disulfide 143144 inhibited the ciliate Oxvtricha and the microalga Chlamydomonas. Based on these initial tests, a range of 5 145concentrations was selected to find an optimal concentration 146 147to eradicate the ciliate Oxytricha with minimal impact on the productivity of the Chlamydomonas culture: 2.5, 5, 7.5, 10, 148 and 20 mg  $L^{-1}$  garlic oil and 7, 14, 36, 73, and 146 mg  $L^{-1}$ 149 diallyl disulfide. Chlamydomonas cultures with and without 150Oxytricha contamination were used as negative and positive 151controls, respectively. The experiments were carried out in 152100 mL reactors aerated with sterile-filtered air and gently 153mixed at 10 rpm (irradiance and temperature were similar as 154for maintenance of stock cultures). The initial concentration of 155microalgae was  $4.4-8 \times 10^6$  Chlamydomonas cells mL<sup>-1</sup> (cor-156responding to OD<sub>750</sub> of 0.18-0.3), and the initial abundance of 157the ciliate Oxytricha was 25 cells mL<sup>-1</sup>. Each treatment was 158prepared in triplicate. The garlic extract and diallyl disulfide 159160 were added to the Chlamydomonas cultures immediately after addition of the ciliates. Growth of Chlamydomonas was mon-161162itored spectrophotometrically (OD750), while Oxytricha abun-163 dance was monitored microscopically in a 1-mL subsample that was preserved with formaldehyde (5%). The cultures 164165were monitored daily for 5 days.

#### 166 Chemical composition of garlic extract

167 The chemical composition of the garlic extract was determined using gas chromatography linked to mass spectrometry 168(GC-MS). GC-MS analysis was carried out on a Thermo 169 170Scientific Trace 1310 system coupled to an ISQ LT Quadrupole MS at a constant helium flow of  $1.2 \text{ mL min}^{-1}$ . 171172The column used was a 30 m length  $\times$  0.25 mm internal di-173ameter with a 0.25-µm DB5-MS film capillary column. The garlic oil was diluted to 1% in dichloromethane, and 0.5 µL 174was injected in a split mode of 1:10. The injector temperature 175and the ion source temperature of detector were 280 and 176250 °C. The oven temperature was 35 °C. Then, at 1 min a 177 $12 \,^{\circ}\text{C} \,^{\text{min}^{-1}}$  ramp, the temperature was raised up to 330  $\,^{\circ}\text{C}$ , 178179which was maintained for 4.3 min. Quantification of diallyl disulfide in the extract was carried out against a pure standard. 180

#### 181 Data analysis

182 Two-way ANOVA was used to test for the independent and 183 interacting effects of the garlic oil and diallyl disulfide and 184 presence/absence of the ciliate *Oxytricha* on the optical den-185 sity of *Chlamydomonas* in the cultures after 5 days. One-way 186 ANOVA was used to evaluate the effect of the garlic extract and diallyl disulfide concentration on the abundance of ciliates187after 1 day. Tukey's HSD post hoc test was used for pairwise188comparisons between samples. All statistical analyses were189carried out using R.190

The toxicity of the garlic extract and the active compound 191 diallyl disulfide was expressed as 1-day LD<sub>50</sub> for the ciliate 192Oxytricha and microalga Chlamydomonas. LD<sub>50</sub> is the con-193 centration at which a chemical is lethal to 50% the exposed 194individuals of a population during a specific period of time. 195LD<sub>50</sub> values were determined from a linear regression plot 196using the probit analysis method, as described by Finney 197 (1952). 198

#### Results

In the positive control without garlic oil and without the ciliate 200 Oxytricha, biomass of the microalga Chlamydomonas in-201creased from an initial OD<sub>750</sub> of 0.2-0.3 to about 0.7 after 202 5 days (Fig. 2; Table 1). In the negative control treatment that 203was contaminated with the ciliate Oxytricha, the abundance of 204Oxvtricha increased exponentially over time from an initial 205abundance of 25 to 270 cells mL<sup>-1</sup> at the end of the experi-206 ment, corresponding to a growth rate of  $0.9 \text{ day}^{-1}$ . Biomass of 207Chlamydomonas in the negative control treatment increased 208initially but started to decline between days 3 and 4, and the 209 final OD<sub>750</sub> of the negative control treatment was significantly 210lower than in the positive control treatment (Table 1). The 211observed impact of ciliate contamination on the 212Chlamvdomonas cultures is comparable with the impact of 213ciliates on microalgal productivity reported in other studies 214(e.g., Moreno-Garrida et al. 2001; Kim Hue et al. 2018). 21506

When garlic oil was added to a Chlamvdomonas culture 216that was contaminated with the ciliate Oxytricha, the ciliate 217was eradicated from the microalgal cultures within 1 day 218when the garlic oil dose was 5 mg  $L^{-1}$  or higher (Fig. 2). 219The estimated 1-day LD<sub>50</sub> for garlic oil to the ciliate 220Oxytricha was 3 mg  $L^{-1}$  (Table 2). The growth of 221*Chlamydomonas* was only affected at a dose of 20 mg  $L^{-1}$ 222or higher and the corresponding LD<sub>50</sub> for garlic oil to 223*Chlamydomonas* was 58 mg  $L^{-1}$ . Garlic oil is thus about 19 224times more toxic to the ciliate Oxytricha than to the microalga 225Chlamydomonas. Because of this large difference in toxicity 226of garlic oil to Oxytricha and Chlamydomonas, garlic oil 227added at a dose of 5, 7.5, or 10 mg  $L^{-1}$  could be used to 228completely remove Oxytricha from a Chlamydomonas culture 229without significantly influencing the productivity of that 230culture. 231

GC-MS analysis indicated that the garlic oil used in our 232 experiments contained mainly polysulfides (Table 3). The 233 most important polysulfides were diallyl disulfide (28%) and 234 diallyl trisulfide (18%). We tested the activity of the most 235 abundant compound present in the garlic oil, being diallyl 236

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Fig. 2 Effect of garlic oil and diallyl disulfide on the growth of Chlamvdomonas culture without Oxvtricha (top), on the growth of Chlamvdomonas in a ciliatecontaminated culture (middle), and on the growth of the ciliate Oxvtricha in the contaminated Chlamvdomonas culture. The extract/chemical were tested at 5 different concentrations and compared with a control treatment (no addition of the chemical). Mean  $\pm$  SD of three biological replicates is given for each treatment. Treatments with the same letter are not significantly different from each other, while treatments with different letters are different from others, according to Tukey's HSD post-hoc test



237disulfide, against Oxytricha and Chlamydomonas. All con-238centrations of diallyl disulfide tested (7 to 146 mg  $L^{-1}$ ) result-239ed in eradication of the ciliate Oxytricha from the240Chlamydomonas cultures, although the lowest concentration241tested resulted in disappearance of the ciliate only after 4 days.242The corresponding 1-day LD<sub>50</sub> for diallyl disulfide to

Oxytricha was 8 mg L<sup>-1</sup> (Table 2). Diallyl disulfide was lethal243to Chlamydomonas at a concentration of 146 mg L<sup>-1</sup> and244reduced the growth at 73 mg L<sup>-1</sup>, but lower concentrations245did not have a significant effect on the growth of this alga. The246corresponding LD<sub>50</sub> for diallyl disulfide to Chlamydomonas247was 117 mg L<sup>-1</sup>. At a concentration between 7 and 36 mg L<sup>-1</sup>,248

Q7 t1.1 Table 1 Results of ANAOVA analyses to test the effect of the garlic extract and its active compound diallyl disulfide on contamination by the ciliate Oxytricha in Chlamydomonas cultures. Two-way ANOVA was used to test the independent and interacting effects of the extracts/ compounds and the ciliate Oxytricha on Chlamydomonas biomass

(estimated from  $OD_{750}$ ) after 5 days. One-way ANOVA was used to compare average cell densities of ciliates treated with different concentrations of each extract/compound after 1 day. For each effect tested in the ANOVAs, the degrees of freedom (d.o.f.), *F* value, and *p* value are given

t1.2			Two-way ANOVA			One-way ANOVA	
t1.3			Oxytricha	Extract/ compound	<i>Oxytricha</i> × extract/compound	Oxytricha abundance	
t1.4	Garlic extract	d.o.f.	1	5	5	5	
t1.5		F	92	116	43	199	
t1.6		р	< 0.001	< 0.001	< 0.001	< 0.001	
t1.7	Diallyl disulfide	d.o.f.	1	5	5	5	
t1.8		F	20	270	23	20	
t1.9		р	< 0.001	< 0.001	< 0.001	< 0.001	

t2.1	Table 2	Toxicity of the garlic extract and diallyl disulfide to the ciliate
	Oxytricha	and the microalga Chlamydomonas expressed as 1-day LD50
	values (pp	m). $LD_{50}$ was estimated using a probit regression. The equation

(y = slope x + intercept) is shown for each regression, as well as the  $R^2$  value and the confidence interval (CI). *y* is mortality percentage in probits, and *x* is log10(dose) (ppm)

t2.2	t2.2 LD <sub>50</sub> Oxytricha				LD <sub>50</sub> Chlamydomonas		
t2.3		LD <sub>50</sub> (95% CI)	Regression	$R^2$	LD <sub>50</sub> (95% CI)	Regression	$R^2$
t2.4 t2.5	Garlic oil Diallyl disulfide	3 (2.4–3.8) 8 (6–10)	y = 5.67 x + 2.28 y = 7.29 x - 1.59	0.99 0.99	58 (23–143) 117 (73–188)	y = -0.71 x + 3.2 y = 2.32 x + 0.17	0.31 0.86

diallyl disulfide was able to eradicate *Oxytricha* from the
 *Chlamydomonas* culture without affecting the productivity
 of the microalgal culture (Fig. 2).

#### 252 **Discussion**

253Garlic has been used for medicinal purposes for thousands of 254years (Block 1985). A large number of scientific studies have confirmed the bioactivity of garlic extracts and the 255256organosulfur compounds they contain, including activity 257against protozoan infectious diseases such as Giardia intestinalis (Harris et al. 2000), Entamoeba parasitica Q8 258 (Ankri et al. 1997), and Trypanosoma species (Lun et al. 259260 1994). In this study, we show that essential oil from garlic 261 prepared through steam distillation can also eradicate a ciliate 262 from a contaminated microalgal culture. The 1-day LD<sub>50</sub> for 263the ciliate Oxytricha estimated in this study was only 3 mg  $L^{-1}$ , which is in the lower end of the range of  $LD_{50}$ 264reported for protozoan parasites: 12 mg  $L^{-1}$  for Leishmania 265*tropica* (Mahmoudvand et al. 2016) or 125 to 1000 mg  $L^{-1}$  for **Q9**266 protozoan parasites of poultry (Zenner et al. 2003). This sug-267gests that the algivorous ciliate that was investigated in this 268study is equally or even more sensitive to garlic oil compared 269270to parasitic protozoans.

To be useful to control ciliate contamination in microalgal cultures, it is important that the garlic oil has a high specificity, i.e., it much more toxic to the ciliate than to the microalgae. A

t3.1 **Table 3** Analysis of the chemical composition of the garlic extract using GC-MS. Compounds that were detected but not quantified are indicated as "nq"

Compounds	Percent
Diallyl disulfide	28
Diallyl trisulfide	18
Diallyl tetrasulfide	1.9
Allyl isopropyl disulfide	nq
Allyl propyl trisulfide	nq
1-Allyl-3-(prop-1-en-1-yl) trisulfide	nq
1-Allyl-3-(2-(allylthio) propyl) trisulfide	nq

low specificity has been reported for many other chemicals 274that are used to control of biological contaminants in 275microalgal cultures (e.g., Karuppasamy et al. 2018; Kim 276Hue et al. 2018). Although the garlic oil was also toxic to 277*Chlamydomonas*, the 1-day  $LD_{50}$  of the garlic extract was 27819 times higher for Chlamydomonas than for Oxytricha. As 279a result, a relatively wide range of garlic oil concentrations (5 280to  $10 \text{ mg L}^{-1}$ ) could be used to eradicate the ciliate from the 281microalgal cultures without significantly reducing the produc-282tivity of the culture. Addition of a higher dose (20 mg  $L^{-1}$ ) 283resulted in a decline in productivity, but not in a complete 284crash of the Chlamydomonas culture. Only one previous study 285investigated the biological activity of garlic oil towards a 286microalga: Zhou et al. (2008) explored the use of garlic oil 287to control different species of red tide microalgae and reported 288a lethal dose of 800 mg  $L^{-1}$ , which is much higher than the 289lethal dose for Chlamydomonas. Further research is needed to 290what extent other microalgal species are sensitive to garlic oil. 291

Garlic oil is a widely available and relatively low-cost 292product (about 20 US\$ kg<sup>-1</sup>). The combination of its low cost 293with a low effective dose needed to eradicate ciliate contam-294ination (5 mg  $L^{-1}$ ); the cost to treat contaminated microalgal 295cultures is low, only about 0.1 US\$ per 1 m<sup>3</sup> of culture broth. 296 Because garlic oil is a natural product that is already used 297commercially for pest control in aquaculture (Lee and Gao 2982012) and agriculture (Kimbaris et al. 2009), there are no 299complex regulatory barriers to its application in microalgae 300 cultivation. Garlic oil has a low toxicity (oral LD<sub>50</sub> to rats is 301425 mg kg<sup>-1</sup>) and therefore poses a low risk to workers ad-302 ministering the product (NPIC 2016). The organosulfur com-303 pounds present in garlic oil are volatile and should disappear 304from the culture medium over time (Avato et al. 2000). These 305properties make garlic oil and attractive product for control-306 ling ciliate contamination in microalgal cultures. Yet, further 307 work is needed to test whether garlic oil is equally effective in 308 large-scale cultivation systems as in a laboratory setting. 309

A potential disadvantage of using natural extracts such as 310 garlic oil is that their chemical composition may be variable 311 and will depend on the origin of the raw resource used to 312 prepare the extract as well as the processing conditions 313 (Gara and Hill 2000). The main bioactive compounds in garlic 314 or garlic extracts are always organosulfur compounds derived 315

316 from alliin, a derivative of the amino acid cysteine. During 317 processing of fresh garlic, alliin is converted into allicin by the enzyme alliinase (Block et al. 1985). Allicin can be further **Q1®**18 converted into other organosulfur compounds such as 319320 vinyldithiins, ajoenes, and polysulfides, depending on the extraction conditions used (polarity of solvent, temperature). In 321 322 this study, we used a garlic essential oil that was extracted 323 using steam distillation. The main organosulfur compounds in garlic oil produced by steam distillation tend to be 324 polysulfides (Block et al. 2010), with the most abundant poly-Q1B25 012826 sulfide usually being diallyl disulfide (Munchberg et al. 2007). 327 This is in agreement with our analysis of the garlic oil using GC-MS. Other types of extracts may have a very different 328 chemical composition. The chemical composition of garlic 329 oil may also depend on the genotype, cultivation conditions, 330 331 or storage of the garlic that was used to prepare the essential 332 oil (Martins et al. 2016). Therefore, to ensure reproducible 333 results, it is important to obtain garlic oil from the same sup-334plier and analyze the chemical composition of the products.

When the activity of the dominant compound in the garlic 335oil extract, diallyl disulfide, is compared to the activity of the 336 crude extract, it is clear that the pure diallyl disulfide to the 337 ciliate Oxytricha (1-day  $LD_{50}$  of 8 mg  $L^{-1}$ ) has a lower toxic-338 ity than the crude garlic oil (1-day  $LD_{50}$  of 3 mg  $L^{-1}$ ). Based 339on this result, it does not make sense to use the pure chemical 340 341diallyl disulfide, especially given the higher cost of diallyl disulfide compared to garlic oil (> 50 US kg<sup>-1</sup>). This result 342 also implies that diallyl disulfide is not the main active com-343 pound in the garlic extract and that other compounds must 344 have a higher activity. The second most abundant chemical 345in the garlic extract was diallyl trisulfide. It is known that 346 347 polysulfides with a higher number of S atoms such a diallyl trisulfide tend to have a higher bioactivity than mono- or 348 disulfides (Munchberg et al. 2007). Further research will be 349 needed to identify the main active compound in garlic oil 350351responsible for the activity against the algivorous ciliate Oxvtricha. 352

353These preliminary experiments do not provide any insight into the mode of action of the garlic oil extract. The 354355polysulfides that are present in garlic oil are reactive mole-356 cules that can oxidize thiol groups on proteins and therefore 357disturb protein activity within the cell (Munchberg et al. 2007). All organisms contain proteins with thiol groups and 358 359should therefore be sensitive to thiol oxidation by polysulfides, but some organisms may be protected from thiol 360 oxidation by the presence of high concentrations of glutathi-361 **013**62 one, which can reduce thiol bonds. Ankri et al. (2007) ascribed the higher sensitivity to polysulfides of Trypanosoma 363 parasites compared to their host to lower glutathione levels in 364the parasite compared to the host cells. Differences in sensi-365 366 tivity of the ciliate to the garlic oil compared to the microalga 367 may also be related to differences in the uptake of polysulfides. Polysulfides are polar compounds (Munchberg 368

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et al. 2007) that might pass more easily through the simple369cytoplasm membrane of the ciliate cells than through the more370complex cell wall of the microalgal cells.371

In conclusion, our results indicate that low doses of garlic 372 oil (mg  $L^{-1}$  range) are capable of eradicating a ciliate contam-373 inant from a microalgal culture. While the ciliate Oxytricha 374and the microalga Chlamvdomonas were used as a model 375 system, further research should confirm whether garlic oil is 376 also effective towards other ciliates and possibly other 377 microalgal grazers and in cultures of other species of 378microalgae. Further work should also elucidate which chem-379 ical is responsible for the activity of garlic oil towards ciliates 380 and explore whether other product derived from garlic may 381 have higher activity (e.g., dry garlic powder, polar garlic ex-382 tracts). The low cost of the product, the low dose needed, and 383 low toxicity to humans make garlic an attractive product for 384 controlling contamination in microalgal culture. 385

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Authors' contributions Nguyen Thi Kim Hue designed and performed 391the experiments, collected and combined data, analyzed statistics and 392interpreted the data, wrote the whole manuscript, reviewed, and edited 393 394the manuscript for important intellectual contents. Koenraad Muylaert obtained the funding, conceived the research and designed the experi-395ments, checked statistical analysis and interpreted the data, reviewed, 396 and developed the whole manuscript for important intellectual contents. 397 Dries Vandamme also contributed to the design of the experiments and 398 was responsible for the GC-MS analysis of the plant extracts. All authors 399read, reviewed, and approved the final manuscript. 400

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