Potential of garlic oil to control biological contamination of Chlamydomonas cultures by the ciliate Oxytricha

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Abstract Facilities for the production of microalgal biomass often suffer large losses in productivity as a result of biological contamination of cultures by ciliates, unicellular protozoans that feed on microalgae. Garlic oil is a low-cost natural product that is known to be active 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 (5–10 mg L^{-1}) were capable of eradicating the ciliate *Oxytricha* from a contaminated *Chlamydomonas* culture within 1 day without influencing the productivity of the *Chlamydomonas* culture. The LD_{50} of garlic oil to the ciliate (3 mg L^{-1}) was 19 times lower than the LD_{50} to the microalgae, which implies a low risk to the microalgal culture in case of overdosing. Analysis of the garlic oil indicated that it was composed mainly of polysulfides, with the main compound being diallyl disulfide. Diallyl disulfide had a lower toxicity to the ciliate (LD_{50} 14 mg L^{-1}) than garlic oil, indicating that diallyl disulfide is not the main active compound in garlic oil against the ciliate. Because garlic oil has a low cost, is already approved for use in agri- and aquacultures, has a low toxicity to humans, and is biodegradable, it may offer a sustainable solution to control biological contamination by ciliates in microalgal cultures.

Keywords separated by ' - ' *Chlamydomonas* culture - Oxytricha contamination - Biological pesticides - Ciliate control - Microalgal 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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Abstract

Facilities for the production of microalgal biomass often suffer large losses in productivity as a result of biological contamination of cultures by ciliates, unicellular protozoans that feed on microalgae. Garlic oil is a low-cost natural product that is known to be active 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 (5–10 mg L^{-1}) were capable of eradicating the ciliate *Oxytricha* from a contaminated *Chlamydomonas* culture within 1 day without influencing the productivity of the *Chlamydomonas* culture. The LD_{50} of garlic oil to the ciliate (3 mg L^{-1}) was 19 times lower than the LD_{50} to the microalgae, which implies a low risk to the microalgal culture in case of overdosing. Analysis of the garlic oil indicated that it was composed mainly of polysulfides, with the main compound being diallyl disulfide. Diallyl disulfide had a lower toxicity to the ciliate (LD_{50} 14 mg L^{-1}) than garlic oil, indicating that diallyl disulfide is not the main active compound in garlic oil against the ciliate. Because garlic oil has a low cost, is already approved for use in agri- and aquacultures, has a low toxicity to humans, and is biodegradable, it may offer a sustainable solution to control biological contamination by ciliates in microalgal cultures.

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

Introduction

Microalgae are attracting worldwide attention as a novel feedstock for the production of biofuels as well as high-value products (Borowitzka 2013). Production of microalgae today is mostly done in open cultivation systems or raceway ponds, but because these are open to the atmosphere, these systems are highly susceptible to biological contamination by pest species such as parasites, weed microalgae and grazers (Wang et al. 2013; Day et al. 2017). These pest species have high growth rates and can cause a complete crash of the culture in a matter of days (Peng et al. 2015). Hence, control of biological contamination is one of the key challenges to develop reliable large-scale production of microalgal biomass.

An important class of biological contaminants is the ciliates: protozoa that feed on microalgae. Several methods have been proposed to control ciliate contamination in microalgal cultures (for a recent overview, see Day et al. 2017). Historically, low doses of formaldehyde have been used to control ciliate contamination (Rothbard 1975) but the use of formaldehyde as a biocide is prohibited in Europe (European Commission 2011). Ciliates can be controlled by a combination of ammonium addition and a pH increase to raise the concentration of free ammonia, but this approach often results in a decline in the productivity of the microalgal culture because free ammonia is not only toxic to ciliates but also to microalgae (Karuppasamy et al. 2018). Inhibitors that are specific to protozoans such as quinine sulfate have been used with success but would be expensive to apply at scale (Moreno-Garrido and Cañavate 2001). Pulsed electric field treatment can be used to kill ciliates but its use is restricted to freshwater microalgae (Kempkes 2017).

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

We used the ciliate *Oxytricha* and the microalga *Chlamydomonas* as a model system to test the potential of garlic for control of ciliate contamination a protozoan grazer of microalgae. *Oxytricha* is frequently reported as a biological contaminant in outdoor microalgal mass cultures (Cho et al. 2017; Wang et al. 2017). We tested a garlic oil extract obtained from an international chemical supplier (Sigma). We also analyzed the chemical composition of the extract and tested the activity of the main active compound present in this extract, diallyl disulfide, to identify the main active compound in the crude garlic extract towards the ciliate *Oxytricha*. Many treatments to control ciliate contamination also inhibit the growth of microalgae when applied at a higher dosage (e.g., Rothbard 1975; Karruppasamy et al. 2017; Kempkes 2017). Therefore, we evaluate the toxicity of garlic oil to the ciliate *Oxytricha* as well as the microalga *Chlamydomonas*.

Materials and methods

Cultivation of microalgae and ciliates

The chlorophyte *Chlamydomonas reinhardtii* SAG 77.81 and the hypotrich ciliate *Oxytricha* sp. that feed on *Chlamydomonas* were used as model systems to evaluate the efficacy of a crude garlic extract and the active compound diallyl disulfide to control ciliate contamination in microalgal cultures. *Chlamydomonas* was maintained in 2 L batch cultures in Wright's cryptophyte (WC) medium (Guillard and Lorenzen 1972) in a temperature-controlled room (20 ± 2 °C) at a light intensity of 80 μmol photons m^{-2} s^{-1} and a light-dark cycle of 16:8 h. Exponential growth phase cultures were used in all experiments. Growth of *Chlamydomonas* cells was monitored spectrophotometrically at optical density 750 nm (OD_{750}) (Griffiths et al. 2011). Optical density was calibrated against gravimetric dry weight measurements (using Whatman GF/F glass microfiber filters) and against cell density (determined microscopically using a Bürker counting chamber).

The ciliate *Oxytricha* was isolated from a eutrophic reservoir close to the KU Leuven campus in Kortrijk, Belgium. Individual cells of the ciliate were picked up using a fine Pasteur pipette, washed several times in sterile WC medium, and then transferred into a *Chlamydomonas* culture. The *Oxytricha* cultures were maintained in 6-well microtiter plates in 3 mL volume cultures. The cultures were renewed every 3 days by transferring 0.5 mL of the ciliate suspension to a well containing 2 mL of fresh WC medium and 0.5 mL of a stationary phase *Chlamydomonas* culture (containing biomass concentration expressed as OD_{750} of 0.7 or 2×10^7 *Chlamydomonas* cells mL^{-1}). Exponentially growing *Oxytricha* cultures with an abundance of about 4000 cells mL^{-1} were used as an inoculum in all experiments. The ciliate was identified as *Oxytricha* based on morphological criteria using Berger (1999) (Fig. 1).

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

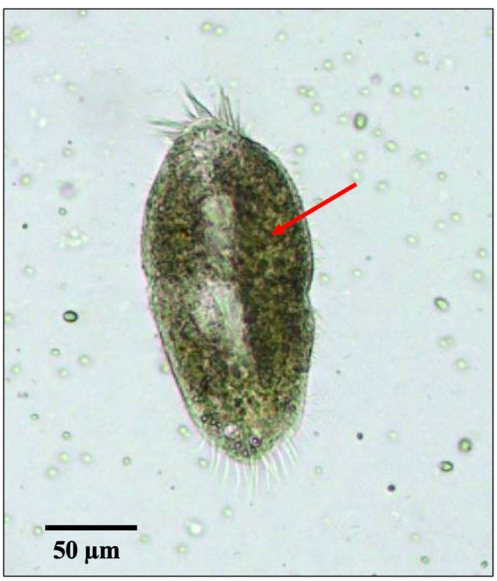

Fig. 1 Photograph of the hypotrich ciliate *Oxytricha* sp. containing *Chlamydomonas* cells in its food vacuoles (red arrow)

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).

Exploratory tests were performed to determine the concentration range at which the garlic oil and diallyl disulfide inhibited the ciliate *Oxytricha* and the microalga *Chlamydomonas*. Based on these initial tests, a range of 5 concentrations was selected to find an optimal concentration to eradicate the ciliate *Oxytricha* with minimal impact on the productivity of the *Chlamydomonas* culture: 2.5, 5, 7.5, 10, and 20 mg L^{-1} garlic oil and 7, 14, 36, 73, and 146 mg L^{-1} diallyl disulfide. *Chlamydomonas* cultures with and without *Oxytricha* contamination were used as negative and positive controls, respectively. The experiments were carried out in 100 mL reactors aerated with sterile-filtered air and gently mixed at 10 rpm (irradiance and temperature were similar as for maintenance of stock cultures). The initial concentration of microalgae was 4.4–8 × 10^6 *Chlamydomonas* cells mL^{-1} (corresponding to OD_{750} of 0.18–0.3), and the initial abundance of the ciliate *Oxytricha* was 25 cells mL^{-1}. Each treatment was prepared in triplicate. The garlic extract and diallyl disulfide were added to the *Chlamydomonas* cultures immediately after addition of the ciliates. Growth of *Chlamydomonas* was monitored spectrophotometrically (OD_{750}), while *Oxytricha* abundance was monitored microscopically in a 1-mL subsample that was preserved with formaldehyde (5%). The cultures were monitored daily for 5 days.

Chemical composition of garlic extract

The chemical composition of the garlic extract was determined using gas chromatography linked to mass spectrometry (GC-MS). GC-MS analysis was carried out on a Thermo Scientific Trace 1310 system coupled to an ISQ LT Quadrupole MS at a constant helium flow of 1.2 mL min^{-1}. The column used was a 30 m length × 0.25 mm internal diameter with a 0.25-μm DB5-MS film capillary column. The garlic oil was diluted to 1% in dichloromethane, and 0.5 μL was injected in a split mode of 1:10. The injector temperature and the ion source temperature of detector were 280 and 250 °C. The oven temperature was 35 °C. Then, at 1 min a 12 °C min^{-1} ramp, the temperature was raised up to 330 °C, which was maintained for 4.3 min. Quantification of diallyl disulfide in the extract was carried out against a pure standard.

Data analysis

Two-way ANOVA was used to test for the independent and interacting effects of the garlic oil and diallyl disulfide and presence/absence of the ciliate *Oxytricha* on the optical density of *Chlamydomonas* in the cultures after 5 days. One-way ANOVA was used to evaluate the effect of the garlic extract and diallyl disulfide concentration on the abundance of ciliates after 1 day. Tukey's HSD post hoc test was used for pairwise comparisons between samples. All statistical analyses were carried out using R.

The toxicity of the garlic extract and the active compound diallyl disulfide was expressed as 1-day LD_{50} for the ciliate *Oxytricha* and microalga *Chlamydomonas*. LD_{50} is the concentration at which a chemical is lethal to 50% the exposed individuals of a population during a specific period of time. LD_{50} values were determined from a linear regression plot using the probit analysis method, as described by Finney (1952).

Results

In the positive control without garlic oil and without the ciliate *Oxytricha*, biomass of the microalga *Chlamydomonas* increased from an initial OD_{750} of 0.2–0.3 to about 0.7 after 5 days (Fig. 2; Table 1). In the negative control treatment that was contaminated with the ciliate *Oxytricha*, the abundance of *Oxytricha* increased exponentially over time from an initial abundance of 25 to 270 cells mL^{-1} at the end of the experiment, corresponding to a growth rate of 0.9 day^{-1}. Biomass of *Chlamydomonas* in the negative control treatment increased initially but started to decline between days 3 and 4, and the final OD_{750} of the negative control treatment was significantly lower than in the positive control treatment (Table 1). The observed impact of ciliate contamination on the *Chlamydomonas* cultures is comparable with the impact of ciliates on microalgal productivity reported in other studies (e.g., Moreno-Garrida et al. 2001; Kim Hue et al. 2018).

When garlic oil was added to a *Chlamydomonas* culture that was contaminated with the ciliate *Oxytricha*, the ciliate was eradicated from the microalgal cultures within 1 day when the garlic oil dose was 5 mg L^{-1} or higher (Fig. 2). The estimated 1-day LD_{50} for garlic oil to the ciliate *Oxytricha* was 3 mg L^{-1} (Table 2). The growth of *Chlamydomonas* was only affected at a dose of 20 mg L^{-1} or higher and the corresponding LD_{50} for garlic oil to *Chlamydomonas* was 58 mg L^{-1}. Garlic oil is thus about 19 times more toxic to the ciliate *Oxytricha* than to the microalga *Chlamydomonas*. Because of this large difference in toxicity of garlic oil to *Oxytricha* and *Chlamydomonas*, garlic oil added at a dose of 5, 7.5, or 10 mg L^{-1} could be used to completely remove *Oxytricha* from a *Chlamydomonas* culture without significantly influencing the productivity of that culture.

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

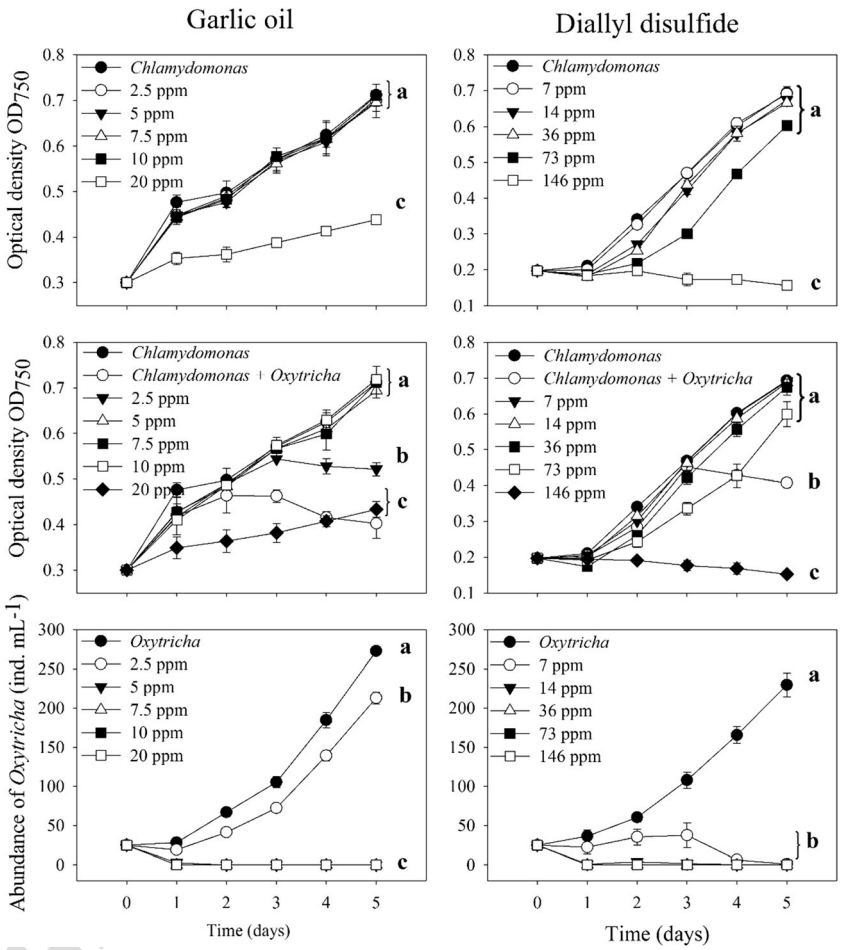

Fig. 2 Effect of garlic oil and diallyl disulfide on the growth of *Chlamydomonas* culture without *Oxytricha* (top), on the growth of *Chlamydomonas* in a ciliate-contaminated culture (middle), and on the growth of the ciliate *Oxytricha* in the contaminated *Chlamydomonas* culture. The extract/chemical were tested at 5 different concentrations and compared with a control treatment (no addition of the chemical). Mean ± 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

disulfide, against *Oxytricha* and *Chlamydomonas*. All concentrations of diallyl disulfide tested (7 to 146 mg L^{-1}) resulted in eradication of the ciliate *Oxytricha* from the *Chlamydomonas* cultures, although the lowest concentration tested resulted in disappearance of the ciliate only after 4 days. The corresponding 1-day LD_{50} for diallyl disulfide to *Oxytricha* was 8 mg L^{-1} (Table 2). Diallyl disulfide was lethal to *Chlamydomonas* at a concentration of 146 mg L^{-1} and reduced the growth at 73 mg L^{-1}, but lower concentrations did not have a significant effect on the growth of this alga. The corresponding LD_{50} for diallyl disulfide to *Chlamydomonas* was 117 mg L^{-1}. At a concentration between 7 and 36 mg L^{-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

			Two-way ANOVA			One-way ANOVA
			Oxytricha	Extract/compound	*Oxytricha* × extract/compound	*Oxytricha* abundance
Garlic extract		d.o.f.	1	5	5	5
		F	92	116	43	199
		p	< 0.001	< 0.001	< 0.001	< 0.001
Diallyl disulfide		d.o.f.	1	5	5	5
		F	20	270	23	20
		p	< 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 LD_{50} values (ppm). 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		LD_{50} *Oxytricha*			LD_{50} *Chlamydomonas*		
t2.3		LD_{50} (95% CI)	Regression	R^2	LD_{50} (95% CI)	Regression	R^2
t2.4	Garlic oil	3 (2.4–3.8)	$y = 5.67\,x + 2.28$	0.99	58 (23–143)	$y = -0.71\,x + 3.2$	0.31
t2.5	Diallyl disulfide	8 (6–10)	$y = 7.29\,x - 1.59$	0.99	117 (73–188)	$y = 2.32\,x + 0.17$	0.86

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

Discussion

Garlic has been used for medicinal purposes for thousands of years (Block 1985). A large number of scientific studies have confirmed the bioactivity of garlic extracts and the organosulfur compounds they contain, including activity against protozoan infectious diseases such as *Giardia intestinalis* (Harris et al. 2000), *Entamoeba parasitica* (Ankri et al. 1997), and *Trypanosoma* species (Lun et al. 1994). In this study, we show that essential oil from garlic prepared through steam distillation can also eradicate a ciliate from a contaminated microalgal culture. The 1-day LD_{50} for the ciliate *Oxytricha* estimated in this study was only 3 mg L^{-1}, which is in the lower end of the range of LD_{50} reported for protozoan parasites: 12 mg L^{-1} for *Leishmania tropica* (Mahmoudvand et al. 2016) or 125 to 1000 mg L^{-1} for protozoan parasites of poultry (Zenner et al. 2003). This suggests that the algivorous ciliate that was investigated in this study is equally or even more sensitive to garlic oil compared to 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 low specificity has been reported for many other chemicals that are used to control of biological contaminants in microalgal cultures (e.g., Karuppasamy et al. 2018; Kim Hue et al. 2018). Although the garlic oil was also toxic to *Chlamydomonas*, the 1-day LD_{50} of the garlic extract was 19 times higher for *Chlamydomonas* than for *Oxytricha*. As a result, a relatively wide range of garlic oil concentrations (5 to 10 mg L^{-1}) could be used to eradicate the ciliate from the microalgal cultures without significantly reducing the productivity of the culture. Addition of a higher dose (20 mg L^{-1}) resulted in a decline in productivity, but not in a complete crash of the *Chlamydomonas* culture. Only one previous study investigated the biological activity of garlic oil towards a microalga: Zhou et al. (2008) explored the use of garlic oil to control different species of red tide microalgae and reported a lethal dose of 800 mg L^{-1}, which is much higher than the lethal dose for *Chlamydomonas*. Further research is needed to what extent other microalgal species are sensitive to garlic oil.

Garlic oil is a widely available and relatively low-cost product (about 20 US$ kg^{-1}). The combination of its low cost with a low effective dose needed to eradicate ciliate contamination (5 mg L^{-1}); the cost to treat contaminated microalgal cultures is low, only about 0.1 US$ per 1 m^3 of culture broth. Because garlic oil is a natural product that is already used commercially for pest control in aquaculture (Lee and Gao 2012) and agriculture (Kimbaris et al. 2009), there are no complex regulatory barriers to its application in microalgae cultivation. Garlic oil has a low toxicity (oral LD_{50} to rats is 425 mg kg^{-1}) and therefore poses a low risk to workers administering the product (NPIC 2016). The organosulfur compounds present in garlic oil are volatile and should disappear from the culture medium over time (Avato et al. 2000). These properties make garlic oil and attractive product for controlling ciliate contamination in microalgal cultures. Yet, further work is needed to test whether garlic oil is equally effective in large-scale cultivation systems as in a laboratory setting.

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

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"

t3.2	Compounds	Percent
t3.3	Diallyl disulfide	28
t3.4	Diallyl trisulfide	18
t3.5	Diallyl tetrasulfide	1.9
t3.6	Allyl isopropyl disulfide	nq
t3.7	Allyl propyl trisulfide	nq
t3.8	1-Allyl-3-(prop-1-en-1-yl) trisulfide	nq
t3.9	1-Allyl-3-(2-(allylthio) propyl) trisulfide	nq

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

When the activity of the dominant compound in the garlic oil extract, diallyl disulfide, is compared to the activity of the crude extract, it is clear that the pure diallyl disulfide to the ciliate *Oxytricha* (1-day LD_{50} of 8 mg L^{-1}) has a lower toxicity than the crude garlic oil (1-day LD_{50} of 3 mg L^{-1}). Based on this result, it does not make sense to use the pure chemical diallyl disulfide, especially given the higher cost of diallyl disulfide compared to garlic oil (> 50 US$ kg^{-1}). This result also implies that diallyl disulfide is not the main active compound in the garlic extract and that other compounds must have a higher activity. The second most abundant chemical in the garlic extract was diallyl trisulfide. It is known that polysulfides with a higher number of S atoms such a diallyl trisulfide tend to have a higher bioactivity than mono- or disulfides (Munchberg et al. 2007). Further research will be needed to identify the main active compound in garlic oil responsible for the activity against the algivorous ciliate *Oxytricha*.

These preliminary experiments do not provide any insight into the mode of action of the garlic oil extract. The polysulfides that are present in garlic oil are reactive molecules that can oxidize thiol groups on proteins and therefore disturb protein activity within the cell (Munchberg et al. 2007). All organisms contain proteins with thiol groups and should therefore be sensitive to thiol oxidation by polysulfides, but some organisms may be protected from thiol oxidation by the presence of high concentrations of glutathione, which can reduce thiol bonds. Ankri et al. (2007) ascribed the higher sensitivity to polysulfides of *Trypanosoma* parasites compared to their host to lower glutathione levels in the parasite compared to the host cells. Differences in sensitivity of the ciliate to the garlic oil compared to the microalga may also be related to differences in the uptake of polysulfides. Polysulfides are polar compounds (Munchberg et al. 2007) that might pass more easily through the simple cytoplasm membrane of the ciliate cells than through the more complex cell wall of the microalgal cells.

In conclusion, our results indicate that low doses of garlic oil (mg L^{-1} range) are capable of eradicating a ciliate contaminant from a microalgal culture. While the ciliate *Oxytricha* and the microalga *Chlamydomonas* were used as a model system, further research should confirm whether garlic oil is also effective towards other ciliates and possibly other microalgal grazers and in cultures of other species of microalgae. Further work should also elucidate which chemical is responsible for the activity of garlic oil towards ciliates and explore whether other product derived from garlic may have higher activity (e.g., dry garlic powder, polar garlic extracts). The low cost of the product, the low dose needed, and low toxicity to humans make garlic an attractive product for controlling contamination in microalgal culture.

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

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