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Removal of *Cryptosporidium parvum* oocysts in low quality water using *Moringa oleifera* seed extract as coagulant

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ABSTRACT

The use of different types of low quality water for irrigation in agriculture is common practice in many countries due to limited freshwater resources. Pathogens may contaminate fruit and vegetables when feces contaminated water is used for irrigation or postharvest processing. A laboratory study was carried out to investigate the effect of a coagulant produced from seeds of the *Moringa oleifera* tree (MO) in reducing *Cryptosporidium parvum* oocysts and turbidity in wastewater and stream water. Glass jars ($n = 60$) containing 500 mL wastewater obtained from the inlet to the primary settling tanks from a Danish sewage treatment plant were spiked with 6.1 x $10^5 \pm 6.2 \times 10^4$ oocysts L^{-1}, while glass jars ($n = 18$) containing 500 mL stream water were spiked with approx. 100, 1000 or 10,000 oocysts. To half of the wastewater and stream water 4 mL L^{-1} of a 5% *w/v* MO seed extract was added, while the remaining water was left untreated. The water was stirred slowly for 20 min and subsequently left to sediment for 15, 30, 45, 60 or 90 min (wastewater) or 60 min (stream water), with three (stream water) or six (wastewater) replicate glass jars representing each time point. In wastewater, MO seed extracts reduced the *C. parvum* oocyst load significantly ($p = 0.03$) by 38% in the interval 15 to 90 min compared to a 0.02% reduction in the untreated wastewater. Furthermore, the number of oocysts L^{-1} was significantly ($p > 0.0001$–$p = 0.041$) reduced in the treated wastewater at all five sampling times compared to untreated wastewater. Likewise, the oocyst loads in the supernatant of MO treated stream water were noticeably lower compared with untreated stream water at all three spikes. The turbidity was reduced to 10.9 ± 0.3 Nephelometric turbidity units (NTU) (i.e. 94.7% reduction) and 13.7 ± 2.1 NTU (i.e. 91.7% reduction) in the treated wastewater and stream water, respectively. In contrast, the turbidity was 55.3 ± 4.4 NTU and 46.2 ± 1.6 NTU in untreated wastewater and stream water, respectively. *M. oleifera* seeds are readily available in many tropical countries where the tree is common, and our results clearly demonstrate that MO seed extract may be used by farmers for treatment of different types of surface water prior to irrigation use. Yet, adding MO seed extract to the low quality water did not successfully remove all oocyst. However, treatment of wastewater with MO seed extract significantly improved the water quality with regard to number of oocysts present and turbidity of the water. Further experiments with addition of higher concentrations of MO are needed to establish whether MO seed extract can be used to obtain safe irrigation water free of *C. parvum* oocysts and other protozoan parasites.

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1. Introduction

An estimated 90% of wastewater in developing countries undergoes no treatment (Corcoran et al., 2010). For example in Kumasi, Ghana, only 8% of the wastewater undergoes some form of treatment (Keraita et al., 2002), while the remaining raw sewage flows to wetlands linked to small streams or is discharged via storm water drains and gutters into surface streams, along which, irrigated vegetable production is practiced (Keraita et al., 2002). An estimated 60% of the formal irrigation (irrigation systems developed and managed by the government) in Ghana is by low quality water collected directly from these streams, rivers or from on-farm ponds containing river or drainage water (Obuobie et al., 2006). The same applies for many other developing countries, where the most commonly used water source for irrigation in urban farming is water from wastewater-contaminated sources (Mateo-Sagasta et al., 2013). Foods produced by irrigation with wastewater are estimated to be consumed by 10% of the world's population (Corcoran et al., 2010) and can pose a significant health risk to consumers and farmers. A major health risk associated with irrigation by feces contaminated waters are pathogens including oocysts of the protozoan parasite *Cryptosporidium*. Protozoan parasites are commonly found in different freshwater sources. For instance, Amoros et al. (2010) demonstrated 47.5 oocysts L^{-1} in water used for irrigation of lettuce in Spain and in Mexico, 98% of irrigation water samples contained *Cryptosporidium*, *Giardia* or both parasites (Chaidez et al., 2005).

Protozoa are transmitted by the fecal oral route, e.g. by consuming (oo)cyst contaminated water, fruit, and vegetables, in particular when produce is consumed raw. *Cryptosporidium* spp. can cause severe or life-threatening gastrointestinal disease in humans as well as animals, in all regions of the world (O'Donoghue, 1995; Okhuysen et al., 1999). The importance of irrigation water as a source of *Cryptosporidium* contamination is underlined by findings of oocysts on vegetables irrigated with low quality water. A study determining the level of *Cryptosporidium*-contamination on 496 vegetable samples from 115 farms around Tehran, Iran, found that 6.6% of the samples were contaminated with *Cryptosporidium*. The irrigation water was associated with the contamination rate, and the *Cryptosporidium* contamination was 33.3% higher when wastewater rather than well water was used for irrigation (Ranjbar-Bahadori et al., 2013). In Ghana, *Cryptosporidium* oocysts were found on 43% of freshly picked lettuce samples from three farms where the irrigation water originated from a nearby stream receiving untreated wastewater from the city of Kumasi (Petersen et al., 2014). In Spain, 63% of lettuce irrigated with water from a wastewater-fed irrigation canal contained *Cryptosporidium* (Amoros et al., 2010).

Cryptosporidium is likely to survive in or on moist food for months and are infectious at low dosages (Okhuysen et al., 1999). A high degree of oocysts removal is therefore required if contaminated water is to be used safely in irrigated agriculture. Low quality water is usually characterized by high turbidity. For example in Ghana, turbidity levels at approximately 200 and 791 nephelometric turbidity units (NTU) have been reported in water used for irrigation (Keraita et al., 2008; Petersen et al., 2014); and high turbidity has been shown to correlate positively with pathogen levels in water (Dorner et al., 2007; Nnane et al., 2011). Thus, turbidity reduction is an important quality parameter when evaluating the effect of wastewater treatment, and turbidity reduction is expected to correlate with reduction of *Cryptosporidium* oocyst levels in low quality water as previously observed for helminth eggs (Sengupta et al., 2012b). Turbidity removal is generally achieved using chemical coagulants, but in recent years, there has been a resurgence of interest in the use of natural materials for water treatment due to cost and associated health and environmental concerns of organic polymers and inorganic chemicals commonly used as coagulants (Ghebremichael and Hultman, 2004). Among plant materials, *Moringa oleifera* (MO) seeds have shown promising qualities as effective coagulants for water treatment (Katayon et al., 2006).

M. oleifera is a non-toxic, tropical plant (Grabow et al., 1985), widespread in the tropical belt (Price, 1985). It is a source of vegetable oil and medicine, and is even consumed as a vegetable (Foidl et al., 2001). *M. oleifera* seeds used as coagulants have been documented to remove 80%–99% turbidity both in raw waters and in synthetic turbid waters (Muyibi and Evison, 1995; Ndabigengesere et al., 1995; Muyibi et al., 2002), although the effect is minor in low turbid water (Muyibi and Evison, 1995).

Sedimentation of particles in water occur naturally, but the sedimentation is very slowly for some small particles, e.g. *Cryptosporidium* oocysts have a sedimentation velocity of only 0.35 μm s^{-1} in Hank's buffered salt solution (HBSS) at 23 °C (Medema et al., 1998). Numerous studies have proven the efficiency of MO seed extract in removing suspended material (Ndabigengesere and Narasiah, 1998a; Ndabigengesere and Narasiah, 1998b; Raghuwanshi et al., 2002) and microorganisms by increasing the sedimentation speed (Madsen et al., 1987; Olsen, 1987; Sengupta et al., 2012b). However, studies on the effect of removal of *Cryptosporidium* oocysts are lacking. The aim of the present study was to use laboratory experiments to assess the ability of MO seed extract to reduce the number of *Cryptosporidium* oocysts in turbid water and simultaneously lower the turbidity.

2. Materials and Methods

2.1. Cryptosporidium parvum oocysts

One week prior to the experiment, *C. parvum* oocysts were concentrated as earlier described (Petersen et al., 2012) from feces of a naturally infected Holstein calf from a Danish farm where the parasite was previously diagnosed. The oocyst concentration in the batch was established by quantifying the oocysts in 10 replicate samples of 100 μL by immunofluorescence microscopy. *Cryptosporidium* oocysts were identified to the species level by polymerase chain reaction (PCR) amplification and partial sequencing of the small subunit ribosomal gene (18S SSU rDNA locus) and the 70-kDa heat shock protein gene (*hsp70*) (Langkjaer et al., 2007).

2.2. Moringa oleifera (MO) seed extract

Seeds of MO were obtained from a local market in the Kumasi region in northern Ghana. The seeds were removed from the pods, transported to Denmark and stored under dry and dark conditions at room temperature for approximately 1 month before use.

The husk covering the seeds was manually removed and the kernels ground to powder using a kitchen mortar. The MO powder (5% weight per volume (*w*/*v*)) was mixed with tap water, and stirred at 300 rpm for 30 min at room temperature using a magnetic stirrer (Multipoint HP15, Variomag, Florida, USA). The suspension was filtered through a 15 μm nylon membrane and the filtrate extract used as a coagulant. To prevent aging effects (Katayon et al., 2006), a fresh solution was prepared the same day as it was used.

2.3. Characterization of water types

The water used to model low quality irrigation water was either wastewater from a Danish treatment plant or water from a local stream. The wastewater was obtained from the inlet to the primary settling tanks at the wastewater treatment plant "Lynetten I/S", Copenhagen in June 2012 (Table 1). The wastewater was decanted into a 60 L container and stirred prior to decanting into the experimental jars. The stream water was collected from a local stream in Fredensborg, Denmark (Table 1). The stream water was decanted into a 60 L container, stirred thoroughly and left at room temperature for 24 h for large soil particles to settle. Then, the supernatant was decanted into a clean 60 L container, creating a stock solution of polluted water, which was stirred prior to decanting into the experimental jars.

2.4. Experimental design

The study consisted of two parts; the first part assessed the effect of time on *C. parvum* oocyst reduction following addition of MO seed extract and was performed in wastewater. The second part assessed the effect of MO seed extract on flocculation and sedimentation of different concentrations of *C. parvum* oocysts. However, due to heavy rain prior to the second experiment, the turbidity in the wastewater from the Danish treatment plant was lower than in the first part of the experiment. Hence, stream water from a local stream was used as an alternative.

2.4.1. Effect of time and treatment with MO seed extract on C. parvum oocyst reduction in wastewater

The flocculation and sedimentation effect of MO seed extract on *C. parvum* oocysts over time was carried out in wastewater. The added volume of MO seed extract was based on results from previous studies (Madsen et al., 1987; Lea, 2010; Sengupta et al., 2012a) along with the guideline by Lea (2010) stating that the lowest possible turbidity in water with a turbidity of 150–250 NTU is obtained by treating with 200 mg MO seed powder per liter of water (equaling 4 mL L^{-1} of a 5% *w*/*v* MO seed extract).

Glass jars ($n = 60$) (dimension: height 360 mm and diameter 53 mm) (Duran, class A, TH. Geyer) were filled with 500 mL wastewater, $6.1 \times 10^5 \pm 6.2 \times 10^4$ *C. parvum* oocysts in 1 mL MQ water were added, stirring at 50 rpm at a magnetic stirrer was started and 4 mL L^{-1} of the 5% *w*/*v* MO seed extract was added to half the glass jars ($n = 30$), while the other half acted as controls ($n = 30$). Following 20 min stirring, the glass jars were carefully removed from the magnetic stirrer and left on a table to sediment for 15, 30, 45, 60 and 90 min, respectively. These time points were chosen since a pilot study revealed that no further turbidity reduction occurred beyond 90 min and because 60 min is the standard sedimentation time recommend when treating water with MO seed extract (e.g. (Beltran-Heredia and Sanchez-Martin, 2009; Lea, 2010; Sanchez-Martin et al., 2010) For each time point, six replicate glass jars of treated water and controls were analyzed. At the given time, 10 mL water was collected from the center of each glass jar and the turbidity was measured with a TN-100 turbidimeter (EUTECH Instruments, Singapore). Subsequent to the collection of water for turbidity measurement, 450 mL supernatant was carefully separated from the sediment in the glass jars by removing 100 mL at a time with a 100 mL volumetric pipette, transferring it into a 500 mL

Table 1
Characteristics of the water quality used in the experiment.

Parameter	Wastewater[a]	Stream water
pH	7.3	8.7
Turbidity (NTU)	184–226	150–175
Suspended solids (mg L^{-1})	309[b]	N.D.
Total organic carbon (mg L^{-1})	153[b]	N.D.
Chemical oxygen demand (mg L^{-1})	576[b]	N.D.
Biochemical oxygen demand (mg L^{-1})	327[b]	N.D.
NH_4-N (mg L^{-1})	28,6[b]	N.D.
Total P (mg L^{-1})	7,93[b]	N.D.
Chloride CL^- (mg L^{-1})	300[b]	N.D.
Cryptosporidium oocysts (number L^{-1})	12–24	0

[a] The wastewater was obtained from the inlet to the primary settling tanks at the wastewater treatment plant "Lynetten I/S".
[b] Water quality characteristics were provided by the wastewater treatment plant 'Lynetten', Copenhagen, Denmark.

blue cap bottle. Attention was paid to avoid disturbing the sludge on the bottom. The supernatant was distributed into nine 50 mL centrifugation tubes and the oocysts were enumerated as described in Section 2.5.

2.4.2. Effect of water treatment with MO seed extract on sedimentation of three C. parvum oocyst concentrations in stream water

To examine whether the flocculation and sedimentation effect of MO seed extract on *C. parvum* oocysts is affected by the concentration of oocysts in the water, 4 mL L^{-1} of 5% *w/v* MO seed extract was added to three replicate water samples added either 100 μl, 1 mL or 10 mL ($n = 18$) of an oocyst batch containing 1000 ± 160 *C. parvum* oocysts mL^{-1}. The study was carried out in 500 mL glass jars (dimension: height 360 mm and diameter 53 mm) (Duran, class A, TH. Geyer) filled with stream water and *C. parvum* oocysts. Stirring at 50 rpm was started and 4 mL L^{-1} of the 5% *w/v* MO extract was added to each glass jar. Following 20 min of stirring, the glass jars were carefully removed from the magnetic stirrer and left to sediment for 60 min, with three replicate glass jars for each *C. parvum* concentration. Then, 10 mL water was collected from the center of each glass jar for turbidity measurement with a TN-100 turbidimeter (EUTECH Instruments, Singapore). Subsequent to the collection of water for turbidity measurement, 450 mL supernatant was carefully collected by aspiration (Heto Lab Equipment, Type: Sue 300Q) and transferred into a clean 500 mL bottle. The pump was rinsed with milliQ (MQ) water between each sample collection. Attention was paid to avoid disturbing the sediment on the bottom. The supernatant was distributed into 50 mL centrifugation tubes and the oocysts were enumerated as described in Section 2.5.

2.5. Enumeration of oocysts

The 50 mL centrifugation tubes containing the supernatant of either wastewater or stream water were centrifuged at 1540x*g* for 10 min. The supernatants were discharged leaving sample volumes of 5 mL, which were combined in one tube. The original tubes were subsequently washed with 2 mL MQ water, which were also added to the tube containing the combined samples. The combined sample was centrifuged for 10 min at 1540x*g* and the supernatant removed by aspiration, leaving a 2 mL sample.

Microscopic detection and quantification of *C. parvum* in each water sample using immunomagnetic separation (IMS) was done according to the manufactures instructions (Dynabeads® Anti-*Cryptosporidium* Kit, Life technologies, Nærum, Denmark) except that the magnetic beads were washed with 45 μL 0.01% Tween 20 (polyoxyethylene sorbitan monolaurate) which were added to the tube containing the IMS product. The IMS product was then placed in a well on a 3-well Teflon printed diagnostic slide (Immono-Cell Int., Mechelen, Belgium). The slide was air-dried overnight, fixed with acetone and left to dry for 5 min, before adding 25 μl of anti-*Cryptosporidium* fluorescein isothiocyanate (FITC)-labeled antibody mix (Crypto-CEL IF test, Cellabs, Australia) according to the manufacturer's instruction. The entire well area of the slide was examined using an epifluorescence microscope at either ×200 or ×400 magnification equipped with appropriate filter blocks (519 and 495 nm excitation wavelength for FITC).

2.6. Statistical methods

The mean number of oocysts per MO treated water sample in both experiments was compared with mean number of oocysts in the untreated water samples. Likewise, the turbidity of the supernatants from both treated and untreated wastewater samples were compared. All oocyst counts, as well as turbidity data were transformed by natural logarithm of x (ln X) and analyzed in a linear normal model with random effects. The normality assumption was validated by quantile–quantile plots, and variance homogeneity was validated by a residual plot. The non-significant effects were removed by stepwise backward model reduction on a 5% significance level. The final model for mean oocysts in the wastewater supernatant included sedimentation time and turbidity of the supernatant as covariates; the water type (± treatment) as a response variable; and the interaction water type × time. One of the MO-treated wastewater replicates (60 min) was removed from the study because the oocyst count was almost five times lower (outlier) than observed in the other replicates.

3. Results

3.1. Effect of time and treatment with MO seed extract on C. parvum oocyst reduction in wastewater

Oocyst counts from the 450 mL supernatant of the MO treated and untreated wastewater following different sedimentation times are shown in Fig. 1. The added MO seed extract lowered the oocyst concentration by 38% ($p = 0.03$) in the supernatant over time (15–90 min) in the wastewater, whereas oocysts in the supernatant of untreated wastewater did not settle (0.02%; $p = 0.91$). Hence, the mean oocyst counts at each sedimentation time point were significantly lower in the treated wastewater compared with the untreated wastewater, i.e. with 35% at 15 min ($p = 0.033$), 42% at 30 min ($p = 0.006$), 64% at 45 min ($p = <.0001$), 52% at 60 min ($p = 0.041$) and 60% at 90 min ($p = 0.0004$). However, the addition of 4 mL L^{-1} of 5% MO seed extract did not remove all oocysts from the top 450 mL supernatant within 90 min of sedimentation leaving 3927 ± 1925 oocysts behind. Using the standard linear regression model, the oocysts present in the supernatant in the sedimentation time interval of 15–90 min, the estimated mean time (hours) for all oocysts to settle in the bottom of 50 mL of MO treated wastewater was calculated to 3.6 h. In comparison, the time for all oocysts to settle in the untreated water was 74.6 h.

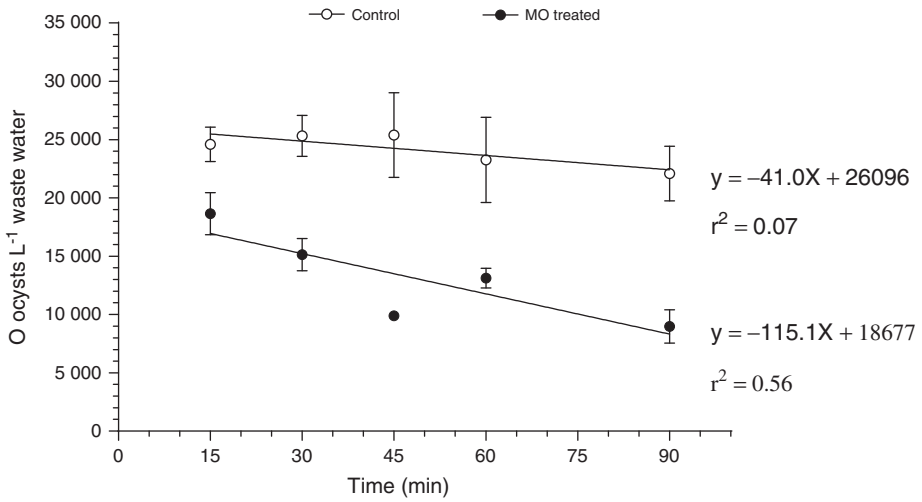

Fig. 1. The effect of time on *Cryptosporidium parvum* oocysts sedimentation. *Cryptosporidium parvum* oocysts sedimentation over time in 500 mL glass jars filled with wastewater and treated with *Moringa oleifera* seed extract (MO treated) and non-treated controls (control). The line represents the linear regression for the time interval 15 min to 90 min.

3.2. Effect of time and treatment with MO seed extract on reducing turbidity

Treatment of wastewater with MO seed extract resulted in 94.7% turbidity reduction following 90 min of sedimentation, corresponding to 10.9 ± 0.3 NTU. In contrast, the final turbidity in the untreated wastewater was 55.3 ± 4.4 NTU, representing 80.3% higher turbidity in the untreated wastewater compared to treated wastewater.

3.3. Effect of water treatment with MO seed extract on sedimentation of three C. parvum oocyst concentrations in stream water

The oocyst counts in the 450 mL supernatant of MO treated and untreated stream water spiked with three concentrations of oocysts following 60 min of sedimentation are illustrated in Fig. 2. The oocyst concentration in the supernatant of MO treated stream waters were 81%, 71% and 88% lower than in the untreated stream water for spikes of 100, 1000 and 10,000 oocysts, respectively. Hence, the difference in percentage oocyst reduction between treated and untreated water was unaffected by spiking dosage.

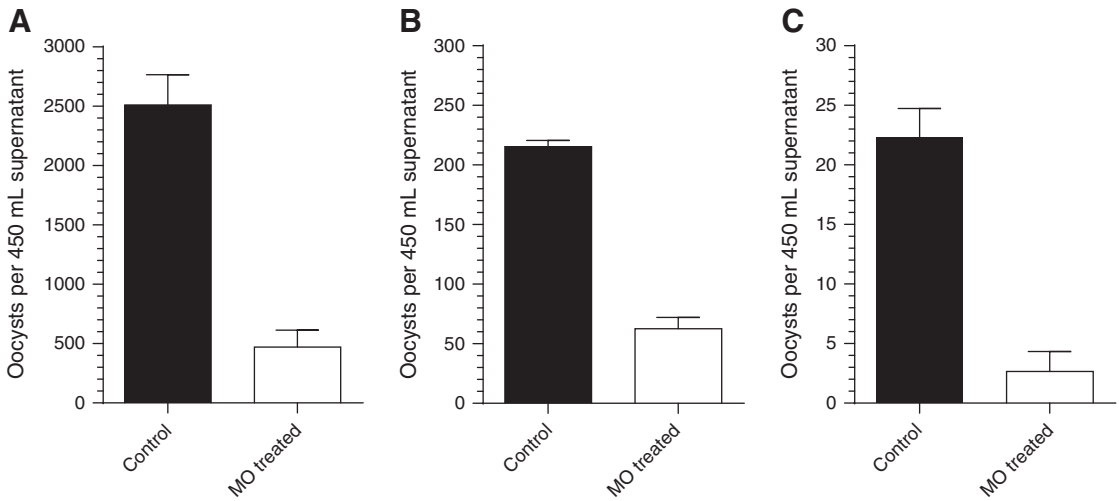

Fig. 2. Sedimentation of three concentrations of *Cryptosporidium parvum* oocyst. *Cryptosporidium parvum* oocyst concentration in the 450 mL supernatant of stream water spiked with approximately 10,000 (A), 1000 (B) or 100 (C) oocysts and treated with *Moringa oleifera* seed extract (MO treated) or non-treated controls (control) in 500 mL glass jars.

The turbidity in the stream water was reduced by 91.7% following 60 min of sedimentation, corresponding to 13.7 ± 2.1 NTU, while the final turbidity in the untreated stream water was 46.2 ± 1.6 NTU, representing 70.4% lower turbidity in the treated stream water compared to untreated wastewater.

4. Discussion

In this laboratory-based study, 60% fewer *C. parvum* oocysts paralleled by 80.3% lower turbidity was obtained following 90 min of sedimentation in wastewater treated with 4 mL L^{-1} of 5% MO seed extract compared with untreated wastewater. This documented effect of increased sedimentation of oocysts in wastewater following treatment with MO seed extract agrees with the results obtained previously for other microorganisms, i.e. helminth eggs, schistosome cercariae and fecal bacteria (Madsen et al., 1987; Olsen, 1987; Sengupta et al., 2012b). Treatment of different types of turbid water with 4 mL L^{-1} of 5% MO seed extract reduced the helminth egg concentration by 94%–99.5% (Sengupta et al., 2012b). Schistosome cercariae were reduced by 90% in artificial Nile water treated with 200 mg L^{-1} MO seeds extract (Olsen, 1987) (equaling 4 mL L^{-1} of a 5% *w/v* MO seed extract), while a fecal bacterial reduction of 1–4 log units was obtained from four types of turbid water treated with 200 mg L^{-1} MO seed extract (Madsen et al., 1987) (representing 4 mL L^{-1} of a 5% *w/v* MO seed extract). *Cryptosporidium* oocysts are small (4–6 μm), have a low specific gravity and are therefore considered unaffected by gravitational settling. Extraction of MO seeds with water releases the active ingredients, the dimeric proteins with a molecular weight of approx. 13 kDa and an iso-electric point of 10–11 (Ndabigengesere et al., 1995). The proteins are water-soluble and positively charged (Ndabigengesere et al., 1995; Broin et al., 2002) and can bind negatively charged particles in the water resulting in floc formation (Ndabigengesere et al., 1995). The net charge of oocysts is negative (Drozd and Schwartzbrod, 1996; Brush et al., 1998) and oocysts can presumably attach to MO seed extract or be incorporated into flocs by binding to the positively charged cationic proteins from MO seed extract. The association with the MO seed extract alters the oocysts "effective" size, changing their settling velocity, which most likely led to the reduced number of oocysts in the supernatant of MO treated wastewater. Nevertheless, in our study 3927 ± 1928 oocysts remained in the 450 mL supernatant of treated wastewater following 90 min of sedimentation. Because the spiking dose of oocysts was relatively high ($6.1 \times 10^5 \pm 6.2 \times 10^4$ oocysts L^{-1}), we first hypothesized that insufficient amounts of the active component of MO seed extract were available to allow complete removal of all oocysts during the formation of flocs. However, the turbidity of the water was reduced significantly and we therefore considered the MO seed extract as fully active. Thus, we studied the effect of treatment with MO seed extract on water containing three lower concentrations of oocysts (100, 1000 or 10,000). Hence, these results did not support our hypothesis, as the percent reduction of oocysts between treated and untreated water was equivalent (81%, 71% and 88%) despite different spiking doses. However, it appears as the percent reduction of oocysts between treated and untreated water was greater in river water (81%, 71% and 88%) than in the wastewater (52%) following 60 min of sedimentation. This could be due to the different water types, different initial turbidity (see Table 1) or different oocyst batches, although the oocysts used in both river- and wastewater originated from the same cattle farm. The partial removal of oocysts from the MO treated turbid water might be due to variations in the oocysts wall structure of the sedimented oocysts and the oocysts remaining in the supernatant. For example, the oocyst viability was not assessed and it is possible that the non-sedimented oocysts had a different viability status than the sedimented oocysts. Oocysts of different viability status and morphologically degenerated oocysts have different density (Young and Komisar, 2005) which possibly also alters the oocysts wall structure. However, further studies are needed to assess if MO seed extract works differently on viable from non-variable oocysts.

Water used for irrigation must preferably be free of oocysts since oocysts might attach to the surface of crops during irrigation or postharvest washing procedures (Monge and Chinchilla, 1995; Amoros et al., 2010), exposing consumers and farmers to infection risk. Risk of acquiring an infection depends on the quantity of viable oocysts, the type of crops and behavior of farmers, food handler and consumers. The survival of oocysts in the environment is affected by factors such as time, humidity, temperature and ultraviolet radiation levels. Ingestion of as few as 10 oocysts can cause cryptosporidiosis in humans (Smith, 1992). Therefore, to minimize the risk of *Cryptosporidium* infection, a high degree of oocysts removal is required if wastewater is to be used safely in irrigated agriculture. Vegetables, especially those eaten uncooked are expected to be safe for consumers. The WHO recommends that a tolerable burden of waterborne disease from consuming wastewater irrigated food is $\leq 10^{-6}$ disability-adjusted life year (DALY) per person per year (WHO, 2006). The pathogenic log-reduction needed to achieve this DALY depends on the exposure scenario (e.g. unrestricted vs. restricted irrigation, type of crops, water type) (WHO, 2006).

Even though we were unable to remove all oocysts from the supernatant, treatment with MO seed extract can substantially improve the wastewater quality by reducing microbial pathogens (Olsen, 1987; Madsen et al., 1987; Ghebremichael and Hultman, 2004; Sengupta et al., 2012b), including *C. parvum* oocysts. Although chemical treatment has been reported to be more effective as a coagulant than MO seed extract, such chemicals may be unavailable and too costly for farmers (Pritchard et al., 2010). *M. oleifera* seeds are easily cultivated and a single tree in a rural area is estimated to provide enough seeds for four families allowing them to treat 20 L water per person per day (Pritchard et al., 2010). The method to extract the coagulant is relatively easy and can be done at low costs. By-products from the extraction can be used as animal feed and fertilizer (Ghebremichael et al., 2005). However, the treatment has several potential limitations. Firstly, treatment of irrigation water with MO seed extract is an expense for the farmer who cannot expect a higher price for the irrigated produce. Secondly, the volume of MO seed extract required to optimally treat water depends on the turbidity (Lea, 2010), and time-consuming individual tests of the water turbidity is required. Thirdly, conflicting information about the correlation between storage time and performance of MO seed extract are reported; Pritchard et al. (2010) documented the shelf life of the seeds to be 18–24 months, while Katayon et al. (2006) demonstrated that coagulation efficiency decreased as storage duration increased (1–5 months).

The land area and costs for establishing and maintaining ponds for water treatment as well as the time and money required to perform treatment with MO seed extract must also be taken into account.

The maximum settling time examined in our study was 90 min, but oocyst sedimentation would probably continue if the settling time was extended resulting in increased oocyst reduction. A time estimate required for all oocysts to reach the bottom of the glass jars was calculated as 3.6 h in treated water and 74.6 h in untreated water. Hence, the oocyst load was reduced 20.7 times faster in wastewater treated with MO seed extract than by natural sedimentation. Our study was based on treatment with a single dose of MO seed extract but further studies including treatment with different doses, different water types and longer settling periods are needed. In conclusion, this laboratory based-study showed that adding 4 mL L^{-1} of a 5% *w*/*v* extract from seed powder of the *M. oleifera* tree significantly reduced the number of *C. parvum* oocysts as compared to natural sedimentation of 90 min in wastewater.

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