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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).