THE EFFECTIVENESS OF WASH WATER TREATMENTS TO PREVENT OR REDUCE THE SPREAD OF PLANT PATHOGENS IN THE HOLLAND MARSH

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In Holland March region, Canada carrots from different fields are washed in the same facility and the wash water can contain potential plant pathogens. Information is scant on the fungal inoculum load and species diversity in treated wash water, which is recycled for irrigation or flows into Lake Simcoe. The present study is undertaken to detect, identify and quantify the fungal pathogens in water from vegetable processing plants and to determine the best method to treat vegetable wash water before it is reused for washing, irrigation or released to flow to drains/creeks entering Lake Simcoe.

This report details the methods and work done in the winter of 2016.

MATERIALS AND METHODS

Site visits began in February, 2016. Five carrot wash units were selected in Holland Marsh area based on the techniques used for treating wash water. Each facility had a different vegetable wash water treatment technology installed. Multiple water samples will be collected from processing plants which employ different techniques for water purification.

Carrot wash facilities selected for wash water sample collection

Wash Facility	Treatments employed	Remarks
Wash Facility-1	3 cell settling pond	
Wash Facility-2	One medium size settling pond	
Wash Facility-3	Vertical filter bags (Geobags) + flocculants	
Wash Facility-4	One large settling pond	Water held for few months
Wash Facility-5	*Geobags* + Red sand filter* + Chitosan filter* +settling pod* + wood chip filter*	Water collected after each step. So 6 samples per wash (*)

Wash water samples (1L) were received from staff of the Holland Marsh Growers Association on weeks of March-14-2016 and March-28-2016. Additional water samples will be received weekly until April 2016 and then monthly. A one liter sample was collected from each step in a wash facility into sterile bottles. Samples were plated the same day or stored at 4°C. When refrigerated storage was done samples were plated within 2 days. A 100 ml of subsample was taken from each 1 L sample for plating.

For initial isolation of fungi from untreated and treated wash water, selective media were used for Pythiaceous fungi (Jeffers and Martin, 1986) and *Fusarium* (Nash and Snyder, 1962). Modified Potato dextrose agar (PDA + 500 ppm chloramphenicol) was used for all other fungi. For Pythiaceous fungi P_5ARP medium containing, 17 g/L cornmeal agar, 5 mg/L pimaricin, 250 mg/L ampicillin, 10 mg/L rifampicin and 100 mg/L PCNB (pentachloronitrobenzene) was used. Nash Snyder medium selective for *Fusarium* contained 15 mg peptone, 1 g KH_2PO_4, 0.5 g MgSO4. 7H2O, 750 mg PCNB, 20 g/L agar, 1000 ppm streptomycin and 120 ppm neomycin per liter.

Two methods were tested for isolating pathogens:

a) For wash water before treatment, plating of water sample at various dilutions ranging from zero to 1:10,000 on fungal growth medium were tested, and will be narrowed down to 1 or 2 dilutions based on the early results. Samples were diluted under aseptic conditions with sterile distilled water. One milliliter of diluted sample was pipetted on media specific for Pythiaceous fungi, *Fusarium* and modified PDA. The water sample was spread evenly on the media and let dry. Each medium was replicated three times. Medium for Pythium isolation was incubated in dark at 25 ^{o}C and that of *Fusarium* and PDA plates were incubated at room temperature (~ 22 ^{o}C)

b) For wash water after treatment, sample dilution followed by plating and concentrating microbes prior to plating were tested. This method was done with water after treatment as the pathogen load was expected to be less in water after treatment. To concentrate the microbes on to a filter, each 50 ml sample was passed through a Durapore5 filter (Millipore, Canada) (Hong *et al*., 2002). Water was drawn through the 5 µm, 47 mm Polyvinylidene fluoride filter using a vacuum pump aspirator (Fischer, Canada). The filter paper was placed in a test tube containing 9 ml 0.09% water agar and vortexed for 60 s at maximum speed. One milliliter of water agar was pipetted to P_5ARP (for Pythiaceous fungi), Nash Snyder (for Fusarium) and modified PDA medium, and each media replicated three times. Filter paper was plated on modified PDA to look for fungal growth to confirm the efficiency of release of pathogen propagules to water agar during vortexing.

Colonies were counted from 5 days after plating. From each plate, 25% of colonies were transferred to PDA for fungal growth and identification.

RESULTS

As of March-31-2016 two sets of samples have been received from wash facilities-1, 2, 4 and 5 and one from wash facility-3. From wash facility-5 only one set of samples could be obtained post red sand and chitosan filters as there was very low water volume from this process.

Wash Facility	**Pathogen**	**Stage of Collection**	**Dilutions Tested**
#1	Pythium, Fusarium and other fungi	Settling Pond In	1:100, 1:200, 1:500, 1:1000, 1:10,000
		Settling Pond Out	Undiluted, 1:100, Concentrated by filtering
#2	Pythium, Fusarium and other fungi	Settling Pond In	1:100, 1:200, 1:1000, 1:10,000
		Settling Pond Out	Undiluted, 1:100, Concentrated by filtering
#3	Pythium, Fusarium and other fungi	Post Wash	1:100, 1:200, 1:500, 1:1000
		Post Geo bag	1:100, 1:200, 1:500, 1:1000
#4	Pythium, Fusarium and other fungi	Settling Pond In	1:100, 1:1000, 1:10,000
		Settling Pond Out	Yet to collect-long hold
#5	Pythium, Fusarium and other fungi	Post Wash	1:100, 1:200, 1:1000, 1:10,000
		Geobag-Water out	Not working
		Post Red sand filter	Undiluted, 1:100, Concentrated by filtering
		Post Chitosan Filter	Undiluted, 1:100, Concentrated by filtering
		Post Settling pond	Undiluted, 1:100, 1:200, Concentrated by filtering
		Post wood chip filter	Undiluted, 1:100, Concentrated by filtering

Based on plating done for 2 set of samples (week of Mar-14-2016 and Mar-28-2016) the following methods/dilutions will be followed for further trials based on optimum fungal growth.

Facility	Treatment	Pythiaceous fungi	Fusarium	Other Fungi
#1	Settling pond in	1:1000	1:500	1:200
	Settling pond out	1:200	1:200	1:200
#2	Settling pond in	1:200	1:200	1:200
	Settling pond out	1:200	1:200	1:200
#3	Post Wash	1:500	1:200	1:200
	Post Geobag	1:100	1:25	1:25
#4	Settling pond in	1:100	1:100	1:200
	Settling pond out	Yet to collect –long hold	Yet to collect –long hold	Yet to collect –long hold
#5	Post wash	1:2000	1:200	1:200
	Post Sand Filter	Filter Concentrate	Filter Concentrate	Filter Concentrate
	Post Chitosan	Filter Concentrate	Filter Concentrate	Filter Concentrate
	Post settling Tank	1:500	1:500	1:500
	Post wood chip filter	1:200	1:200	1:200

The method or dilution was chosen based on colony growth, so that there are sufficient number of colonies per plate with little or no coalescing of adjacent colonies. Pythiaceous fungi were found to be growing even 4 weeks after plating (WAP) from chitosan filter treated and filter concentrated water. Hence plates of Pythiaceous fungi should be monitored for 4 weeks. Colony growth and development in Nash Snyder medium (Fusarium) and modified PDA stopped by 7-10 days after plating.

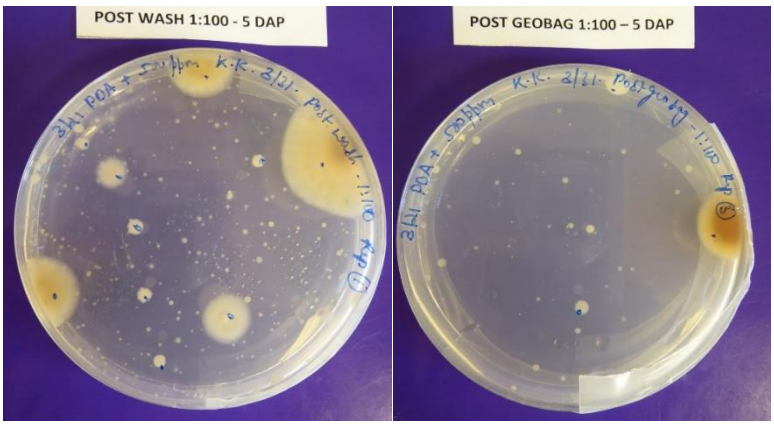

Figure: Sample from wash facility-3, 5 DAP on PDA post wash and post Geobag purification at 1:100 dilution. Pictures from sample plated on week of March-28-2016

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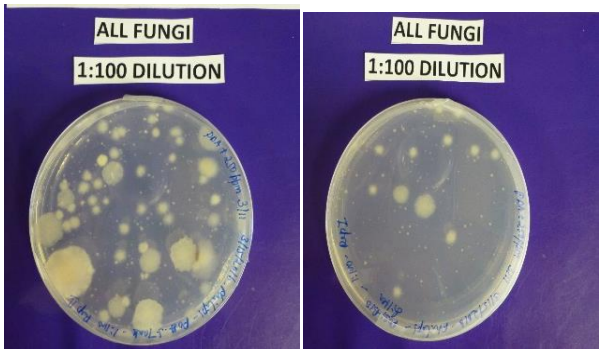

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Figure: Samples from washing facility-5. a) Post wash on PDA 5 DAP (days after plating), b) Pythiaceous fungi post red sand filter 5 DAP, c) Pythiaceous fungi post chitosan filter 5 DAP, d) Pythiaceous fungi post chitosan filter 4 weeks after plating WAP, e) Post settling pond on PDA 5 DAP, f) post wood chip filter on PDA 5 DAP. Pictures from sample plated on week of March-14-2016

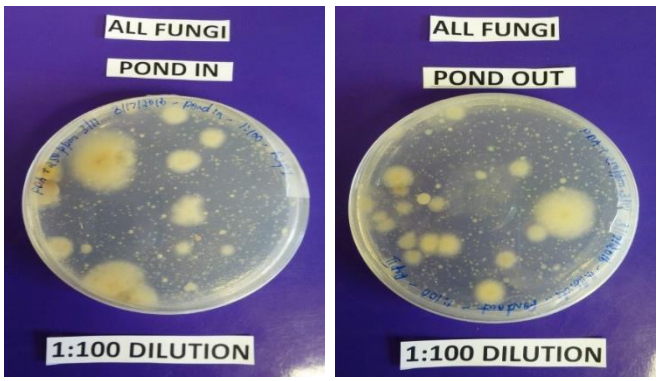

Figure: Samples from wash facility-2 post wash (Pond In) and Post settling pond (Pond Out) in PDA. Pictures from sample plated on week of March-14-2016

ONGOING WORK

Sub culturing of fungal colonies for identification

Taking colony count to determine total colony forming units (CFU) in unit volume of water sample after each treatment.

Inoculation of isolates, especially *Fusarium* in carrot roots to test for pathogenicity.

Testing various dilutions of isolates for petri film plating to identify pathogens based on morphological growth on petri films.

Molecular analysis of select cultures especially Pythiaceous fungi at University of Guelph, Guelph lab.

RESULTS-EXPECTED TIME FRAME

The total colony forming units will be counted after culturing at least 5 sets of samples. Results of CFU/ ml of water and some results on pathogen identification are anticipated in May -2016.

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